Spring 1996

Formation and fate of methyltin compounds in the Great Bay Estuary (New Hampshire)

Anne M. Falke
University of New Hampshire, Durham

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

FORMATION AND FATE OF METHYLTIN COMPOUNDS IN THE GREAT BAY ESTUARY (NEW HAMPSHIRE)

BY

ANNE M. FALKE
Bachelor of Arts, Education, University of Rhode Island, 1981

DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy

in

Chemistry

May, 1996

Dissertation Director
James H. Weber
Professor of Chemistry

This dissertation has been examined and approved.

Dissertation Director, James H. Weber
Professor of Chemistry

Christopher F. Bauer
Associate Professor of Chemistry

Nancy E. Kinner
Associate Professor of Civil Engineering

Richard P. Johnson
Professor of Chemistry

W. Rudolf Seitz
Professor of Chemistry

Feb 5, 1996
Date

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

FORMATION AND FATE OF METHYLTIN COMPOUNDS IN THE GREAT BAY ESTUARY (NEW HAMPSHIRE)

by

Anne M. Falke
University of New Hampshire, May, 1996

Methyltin compounds (MeSn), which are not of anthropogenic origin, are common in estuaries, particularly in the marsh grass *Spartina alterniflora.* During the1989 growing season, *S. alterniflora* leaves collected from three locations in the Great Bay Estuary were analyzed for MeSn and inorganic tin by hydride generation/atomic absorption spectrophotometry (HG-AAS). Total MeSn concentrations showed a sharp increase early in the season, reaching a maximum on May 23, then gradually decreased. Statistical calculations verified that date was a significant contributor to the observed variance, while location was not.

Two years later the seasonal study was expanded to include below ground portions of the plant and the surrounding porewater. HG-AAS analyses on samples collected weekly at Chapman's Landing detected monomethyltin and Sn in all plant parts, with no apparent temporal trend. Concentrations of Sn and monomethyltin were generally higher in roots than in other plant parts. Additionally, there was no

increase in MeSn concentration either concurrent with or following an increase in Sn concentration, indicating that Sn and MeSn are absorbed from the surrounding sediment/porewater and poorly translocated to the remainder of the plant.

Further studies examining the methylation of Sn by decaying *S. alterniflora* and by estuarine water were conducted to ascertain the contribution to MeSn concentrations found in the estuary. Samples of estuarine water spiked with Sn and MeSn were analyzed for Sn and MeSn periodically for four days. Overall Sn concentration decreased while total MeSn concentration increased, indicating methylation. Samples of *S. alterniflora* leaves in estuarine water spiked with Sn were sampled periodically for 120 h. While some conversion of MeSn species could be detected, no net methylation occurred during the time frame studied.

To further elucidate formation of MeSn in the estuary, a hydride generator was coupled to a gas chromatographic mass spectrometry (GC/MS) via cryogenic trapping and thermal desorption. Both sensitivity and precision were improved by cryogenically focusing the analytes at the head of the GC column. This method enabled sensitive and selective speciation of MeSn, and also yielded positive compound identification and the opportunity to use isotopically labeled compounds to trace methylation experiments.

CHAPTER I

INTRODUCTION

The intent of this research was to unravel some of the complexities of the biogeochemical cycling of tin in the environment. Toward that end, we have used instrumentation previously developed for methyltin speciation to conduct a seasonal survey of the Great Bay Estuary (NH), particularly Chapman's Landing, and to examine the role of decaying marsh grass in the methylation cycle. To further investigate the tin methylation cycle in the environment, a speciation method using gas chromatographic mass spectrometry was developed. This will enable researchers to trace isotopically labeled tin through its environmental cycle.

Methyltin compounds (MeSn) are ubiquitous in the environment. They have been found at ng l^{-1} concentrations in seawater (Thompson *et al.*, 1985, Donard *et al.*, 1986, Maguire *et al.*, 1986, Maguire, 1991), and at ng g^{-1}, and higher, concentrations in sediment (Thompson *et al.*, 1985, Donard *et al.*, 1986, Maguire, 1991), macroalgae (Maguire, 1991, Quevauviller *et al.*, 1989, Donard *et al.*, 1987), plants (Maguire, 1991, Wright and Weber, 1991, Francois and Weber, 1988, Francois *et al.*, 1989, Weber *et al.*, 1991), and fish (Maguire, 1991). In fact MeSn may constitute up to 80% of the total tin in water and greater than 90% of the total tin in biota (Short, 1992) The only obvious anthropogenic source for

methyltin is dimethyltin leachate from PVC pipes (Donard *et al.*, 1986). This does not account for concentrations of MeSn found in the environment. Therefore, it is likely that environmental methylation of *Sn* is the major source of these compounds in the environment. A possible schemata for transformation of tin compounds throughout the environment is shown in Figure 1.1.

Biomethylation of tin is of concern because MeSn are much more toxic to marine life and, ultimately, to humans than their inorganic tin precursors. Toxicity of tin compounds increases with the number and size of the alkyl groups attached (Hall and Pickney, 1983). The larger the number and molecular weight of attached alkyl groups, the lower the solubility in water and the greater the solubility in fats. This implies that organotin compounds are more readily bioaccumulated than inorganic tin and may have a tendency to bioconcentrate in certain tissues (Hall and Pickney, 1983). Exposure to methyltin compounds results in morphologically detectable damage to several mammalian organ systems (Hall and Pickney, 1983). MeSn exhibit higher mammalian toxicity and neurotoxicity than any other organotin compound (Hamasaki *et al.*, 1995a). Trialkyltin compounds are the class of organotin compounds which have the greatest biocidal activity in mammals (Means and Hulebak, 1983). Thus, methylation of *Sn* or partially methylated tin in the environment produces a significantly more toxic species.

There are several reasons for emphasizing salt marshes of the Great Bay Estuary and particularly the saltwater cordgrass, *Spartina alterniflora,* in this study. There are *ca.* 4.1 km^2 of salt marsh encompassed in the Great Bay Estuary making this the third most abundant

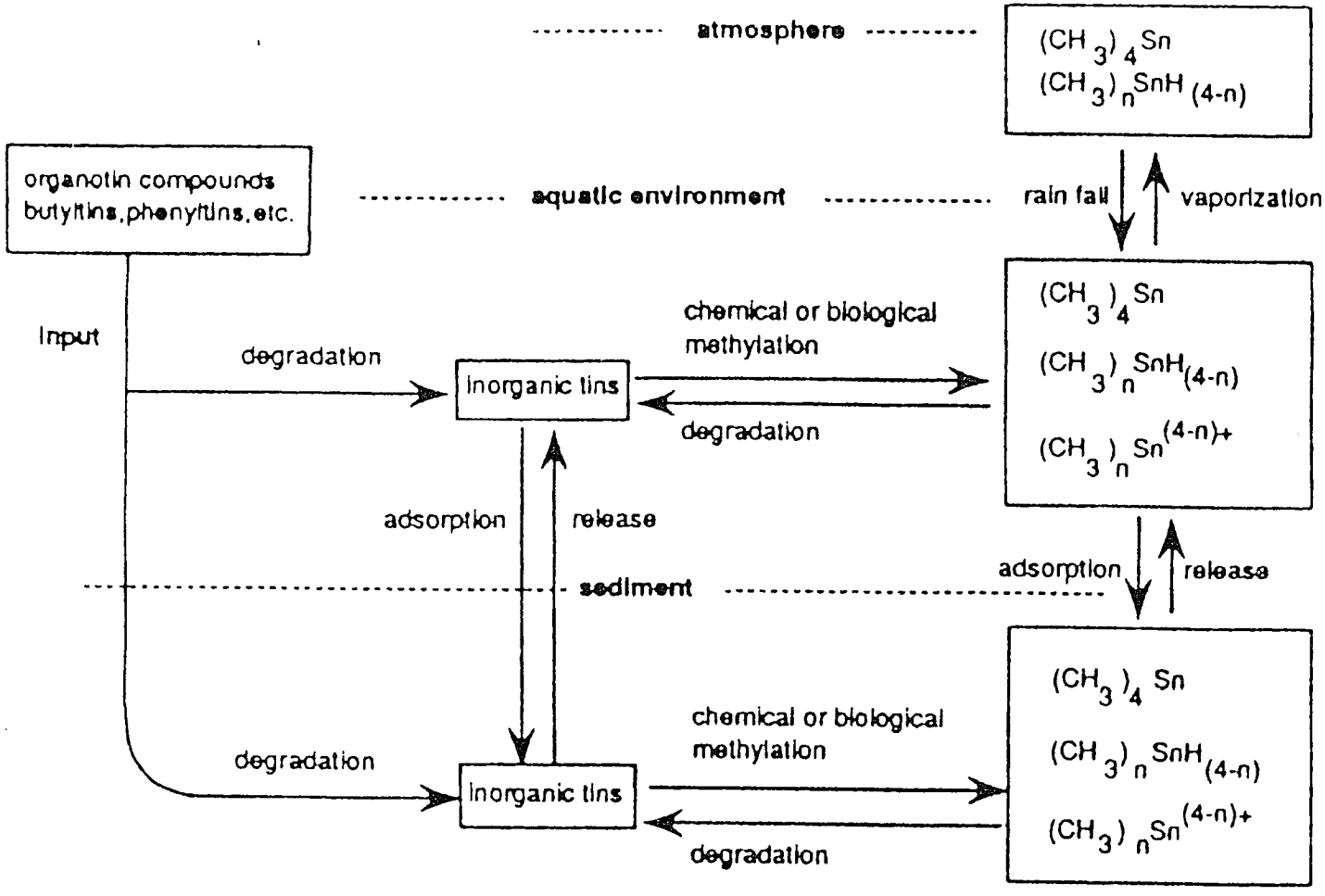

Figure 1.1. Transformations of methyltin in the environment (Hamasaki *et al.*, 1995b).

habitat within the estuary (Short, 1992). *S. alterniflora* is the dominant graminoid of Atlantic coastal salt marshes from Georgia to the Bay of Fundy. It is virtually the only angiosperm found in the intertidal zone of the low marsh (the region between the low and high tide levels) (Shea *et al.*, 1975). Marsh grass ecosystems provide homes, feeding grounds, and breeding grounds for many species of marine organisms and birds. Several species residing within the Great Bay Estuary salt marshes are classified as rare or endangered by the State of New Hampshire (Short, 1992). Relatively large concentrations (470 ng g^{-1}) of monomethyltin ($MeSn^{3+}$) have been detected in the leaves (Weber *et al.*, 1991) of *S. alterniflora*. Decaying *S. alterniflora* has the ability to concentrate metals from the surrounding water column (Alberts *et al.*, 1994). Since *S. alterniflora* constitute a large portion of the organic detritus that fluxes into the Great Bay Estuary each year (Short *et al.*, 1992), it may be an important link in the annual cycling of tin compounds.

Tin Methylation

Based on the relatively high concentrations of MeSn found in some environmental systems (Maguire *et al.*, 1986), especially estuaries (Randall *et al.*, 1986b), and minimal anthropogenic input, there remains little doubt that inorganic tin is being methylated within the environment. Researchers have attempted to isolate systems that contribute to MeSn production. Since high concentrations of MeSn have been found in the leaves of *S. alterniflora*, Weber and Alberts (1990) investigated the possibility that the marsh grass

methylates tin during its growth cycle. They grew *S. alterniflora* hydroponically in tin amended Hoaglund's nutrient solution. At lower concentrations (0.05-0.5 μg Sn ml^{-1}), the plants accumulated inorganic tin from the solution. Most of the tin remained in the roots. At high concentrations (> 1 μg Sn ml^{-1}), the plants appeared to block accumulation of tin. There was no increase in Sn concentration in leaves of any of the inorganic tin amended experiments. There was, however, a significant increase in Me_3Sn^+ concentration in leaves, but not in roots. Two possible explanations for these observations are; (1) tin is taken up by the roots, translocated to the leaves and rapidly methylated there, or (2) tin is taken up by the roots, methylated in the root area and MeSn, but not inorganic tin, is rapidly translocated to the leaves.

Quevauviller *et al.* (1989) observed higher MeSn concentrations in sediments in industrial areas with high inorganic tin concentrations than in non-polluted areas. The anoxic conditions in these sediments favor chemical methylation. Chemical methylation coupled with microbial methylation was most likely responsible for the high MeSn concentrations. In sediments along the Arribida coast (Portugal) they found Me_2Sn^{2+} and Me_3Sn^+ in January, but not in May. This suggests that methylation processes are active in the winter. In May the concentrations of Me_2Sn^{2+} and Me_3Sn^+ decreased. This is accompanied by the occurance of $MeSn^{3+}$, suggesting that $MeSn^{3+}$ originates from demethylation of Me_2Sn^{2+} and Me_3Sn^+.

Donard and Weber (1988) expected that in the presence of decaying algae (*Enteromorpha* sp.), Sn(IV) and Sn(0) would be converted to Me_4Sn. Instead they discovered

that decaying algae mediate production of volatile SnH_4. In addition, $MeSn^{3+}$ was detected at low concentrations (2 - 27 ng ml^{-1}) in the aqueous phase of tin amended solutions, but only at trace levels (0 - 0.2 ng ml^{-1}) in unspiked samples. They detected higher concentrations of MeSn in samples incubated with Sn(IV) than with Sn(0) suggesting a more efficient conversion pathway for Sn(IV) than Sn(0). The major methylation product in experiments spiked with Sn(IV) was $MeSn^{3+}$, while Me_3Sn^+ was prevalent in Sn(0) amended experiments.

Another possible source of MeSn in the environment is input from wastewater treatment plants. Donard *et al.* (1993) found that methylation occurs in the wastewater treatment process. They detected highest MeSn concentrations in the plant influent indicating high methylation rates in raw sewage. The amount of MeSn decreased with each step in the treatment process. Volatile hydrides, SnH_4 and $MeSnH_3$, were found in the anaerobic fermentation steps of activated sludge suggesting an association with methane production.

Anagnostopoulos and Hadjispyrou (1994) examined the effect of increasing Na_2S concentration on methylation of Me_3Sn^+ under biotic and abiotic conditions. In all cases the concentration of Me_4Sn produced in abiotic slurries was between 40 and 47% of that produced in biotic slurries. They also found that increasing sulfide content inhibits methylation, probably because it causes an increase in the pH. They concluded that methylation of Me_3Sn^+ to Me_4Sn is by abiotic transmutation. The higher methylation rates in

biotic slurries were attributed to biotic production of H_2S from Na_2S (effectively reducing the pH) rather than biological methylation.

Many researchers have explored specific pathways to environmental methylation of inorganic tin. Their research focusses on either biotic, *i.e.* by microorganisms, or abiotic methylation. Abiotic methylation includes methylation by chemicals naturally present in the environmental system and methylation by compounds that are byproducts of a biotic process. A summary of the research in this area follows.

Biotic Methylation

The majority of those favoring biotic methylation contend that it occurs primarily in anaerobic sediment layers by *in situ* microorganisms. Ashby and Craig (1988) found that sediments incubated under strictly anoxic conditions produced MeSn. The sites more polluted with inorganic tin produced more MeSn, while sterile sediments produced none. When they attempted to isolate the microorganism responsible for methylation they found that *Pseudomonas* isolated from sediment methylated Sn(II), but not Sn(IV). Another microorganism, *Saccromyces cerivsiae*, was capable of methylating Sn(II) oxalate, sulfide, and amino complexes at a 0.02 - 0.05 % conversion rate. However, incubation with $SnCl_2$ produced no MeSn. Hallas *et al.* (1982) confirm the notion that estuarine microorganisms, especially sediment microflora have the ability to biotransform Sn(IV) to Me_2Sn^{2+} and

Me_3Sn^+ under typical estuarine conditions. In their study, neither sterile controls nor samples poisoned with sodium azide produced MeSn.

Gilmour *et al.* (1987) contend that tin methylation in sediments is primarily a microbially mediated process that favors anaerobic conditions. In one study, they determined that tin methylation occurs in intact estuarine sediments, but not in sediments made sterile through heat treatment (Gilmour *et al.*, 1985). They found that the rate of MeSn production is highest in anoxic sediments implying that anaerobic organisms are primarily responsible for methylation. *Desulfovibrio ssp.* isolated from sediment retained the ability to methylate inorganic tin (Gilmour *et al.*, 1985). Methylation by *Desulfovibrio ssp.* alone occurred at a rate comparable to that in the sediment indicating that tin methylation in the sediment is microbially mediated and that *Desulfovibrio ssp.* is the primary methylating microorganism. In a later study, Gilmour *et al.*, (1987) incubated sediments spiked with inorganic tin under anoxic conditions. Methylation was a slow process, requiring over 60 days and a net methylation of only 0.02% of added tin was attained. $MeSn^{3+}$ was the major MeSn compound formed. Sulfate reducing bacteria isolated from sediment are capable of producing $MeSn^{3+}$ indicating that the methylation process is catalyzed primarily by them. However, addition of sulfide to the culture medium did not catalyze methylation reactions as it does for other metals (Gilmour *et al.*, 1985).

Work by Errecalde *et al.* (1995) also suggests that methylation of inorganic tin by microorganisms is possible. They incubated seven different microorganisms with inorganic

tin in nutrient media. All of the microorganisms studied induced methylation in small proportions, while no MeSn were found in culture media in the absence of microorganisms. In all cases the methylation product was Me_3Sn^+. Additionally, two of the microorganisms studied, *Pseudomonas fluorescens* and *Schizosaccharomyces pombe*, biotransformed monobutyltin to MeSn. The principal transformation products were Me_2Sn^{2+} and Me_3Sn^+.

Abiotic Methylation

Biotic methylation of tin is a very real possibility, however, it is very difficult to verify that it is the only methylation pathway or even the major one. Experimental design generally requires concentrations of inorganic tin that are unusually high relative to ambient concentrations. This tends to favor tin resistant bacteria and may inhibit or even kill others. Also, methods used to sterilize samples (heat or addition of sodium azide) may alter the chemistry of the system.

There are three potential routes of abiotic methylation:

(1) oxidative addition of a methyl cation:

$$Sn(II) + CH_3^+ \rightarrow CH_3Sn(IV)^{3+} \qquad \text{(Equation 1.1)}$$

possible donor - iodomethane (MeI)

(2) addition of a methyl anion:

$$Sn(IV) + CH_3^- \rightarrow CH_3Sn(IV)^{3+} \qquad \text{(Equation 1.2)}$$

possible donor - methylcobalamin ($MeCoB_{12}$)

(3) oxidative addition of a methyl radical:

$$CH_3 + Sn(II) \rightarrow CH_3Sn(III)^{2+} \qquad \text{(Equation 1.3)}$$

followed by further oxidation:

$$CH_3Sn(III)^{2+} \rightarrow CH_3Sn(IV)^{3+} \qquad \text{(Equation 1.4)}$$

possible donor - $MeCoB_{12}$

Several researchers have investigated methylation by environmentally available abiotic methyl donors. A summary of their work follows.

Methylation by oxidative addition of carbocation. Many investigators have attempted to methylate inorganic tin with naturally occurring methyliodide (MeI) under both anoxic and oxic conditions. Rapsomanikis and Weber (1985) reacted Sn (II) with MeI forming methyltin compounds. The relative yield was: $MeSn^{3+} > Me_2Sn^{2+} > Me_3Sn^+$ indicating successive methylation. Craig and Rapsomanikis (1982) concurred, adding that if Mg(0) was present Me_4Sn was also formed. Mg(0) is capable of reducing Sn(II) to Sn(0). MeI is then oxidatively added to Sn(0) yielding MeSn(II)I leading to further oxidative addition and subsequent formation of $Me_2Sn(IV)I_2$. It is important to note that MeSn(II)I has not been isolated and is only a possible intermediate.

$$MeI + Sn(0) \rightarrow [MeSn(II)I] \qquad \text{(Equation 1.5)}$$

$$[MeSn(II)I] + MeI \rightarrow Me_2Sn(IV)I_2 \qquad \text{(Equation 1.6)}$$

Redistribution yields higher order methyltin compounds:

$$2\ Me_2SnI_2 \rightarrow Me_3SnI + MeSnI_3 \qquad \text{(Equation 1.7)}$$

$$2\ Me_3SnI \rightarrow Me_4Sn + Me_2SnI_2 \qquad \text{(Equation 1.8)}$$

The final redistribution reaction is irreversible because the Me_2SnI_2 product redistributes as in Equation 1.7. Reactions of Sn(II) do not produce Me_4Sn because of diminishing yields from the dismutation reaction of the original monomethyl oxidative addition product.

Lee and Weber (1988) found that Sn(II) reacts with MeI producing $MeSn^{3+}$ (up to 13% yield) under strictly anoxic conditions with increased concentrations of Sn(II) and MeI. Data indicate that Sn(II) is rapidly oxidized to Sn(IV) in contact with air. Sn(IV) is not methylated by MeI. However, Sn(IV) reduces to Sn(II) in the sulfidic zone making methylation possible. Therefore, methylation is expected to occur in the anoxic sulfidic zone of the sediment column and only minimally in oxic surface layers. They also found that the presence of fulvic acid inhibits methylation. The possible reasons suggested are: (1) complexation of Sn(II) by ligands, (2) oxidation of Sn(II) by ligands, and (3) binding of MeI by ligands. Interference of the methylation process by fulvic acid suggests that methylation must take place in isolation from fulvic acids and therefore may be an intercellular process.

Manders *et al.*(1984) found that Sn(II) bound to sulfur (SnS) (as is thought to be the case in the sulfidic zone in the sediment) will react with MeI at 60°C giving a 33 % yield of $MeSn^{3+}$. No Me_2Sn^{2+} or Me_3Sn^{+} was found. Reaction of MeI with $SnCl_2$ or SnI_2 at room temperature produced lesser amounts of $MeSn^{3+}$.

Methylation by addition of carbanion. $MeCoB_{12}$ is the only known environmentally available carbanion donor and can methylate metals and metalloids via methyl anion transfer (Ashby and Craig, 1988). However, attempts to methylate Sn(IV) with $MeCoB_{12}$ have been unsuccessful (Fanchaing and Wood, 1981).

Methylation by methyl radical donor. $MeCoB_{12}$ may also function as a methyl radical donor to Sn(II). Fanchaing and Wood (1981) examined methylation of tin chloride salts by $MeCoB_{12}$ under both aerobic and anaerobic conditions at pH 0 to1. In that pH range $MeCoB_{12}$ is in the 'base off' form (*i.e.*, 5,6-dimethylbenzimidazol is protonated and not coordinated to cobalt). Under these conditions Sn(II) was methylated to $MeSn^{3+}$ but no Me_2Sn^{2+} was detected. This reaction follows second order rate law, first order in both Sn(II) and $MeCoB_{12}$. The rate constant for methylation (23°C, 1 M HCl) was 1.04 $M^{-1}s^{-1}$. Sn(II) was not methylated by $MeCoB_{12}$ under strictly anaerobic conditions after 24 h. No MeSn products were detectable in the reaction between Sn(IV) and $MeCoB_{12}$. Conversely, Dizikes *et al.* (1978) found that transfer of a methyl group to Sn(II) by $MeCoB_{12}$ required presence of oxygen or another oxidizing agent (generally aqoucobalamin or Fe(III)). The proposed

methylation sequence was initiated by oxidation of Sn(II) to an Sn(III) intermediate followed by oxidative addition of a methyl radical. In chloride media with excess Sn(II) and Fe(III) the second order rate constant was 1.4 $M^{-1}s^{-1}$.

Ashby and Craig (1991a) found evidence of abiotic methlylation by $MeCoB_{12}$. They demonstrated that tin(II) chloride and tin(II) amino acid complexes can be methylated by $MeCoB_{12}$ under model environmental conditions in the laboratory. Sn(II) is present in significant amounts in anoxic sediments bound to sulfur or nitrogen atoms. Methylcobalamin is demethylated by $SnCl_2$ in acidic to neutral media and 0.01 to 1.0 M chloride concentration. $MeSn^{3+}$ is the product of this reaction. The rate and yield of $MeSn^{3+}$ production is inversely proportional to pH and directly proportional to chloride ion concentration.

Rapsomanikis and Weber (1985) found that maximum yield of total MeSn is achieved through a combination of carbocation and carbanion donors. They attempted methylation of Sn(II) via a carbocation donor (MeI) alone, and in combination with an oxidizing agent (MnO_2) or a macrocyclic carbanion donor ($Me_2Co(N_4)^+$) in 0.1 M KCl solution maintained anaerobically. Reacting Sn(II) with only MeI resulted in a 10.7 % yield of MeSn. Additional presence of MnO_2 decreased the yield to 7.4%, probably due to surface adsorption of Sn(II). Addition of $Me_2Co(N_4)^+$ to the reaction also decreased the yield of $MeSn^{3+}$, but increased the yield of Me_2Sn^{2+} and Me_3Sn^+ indicating further methylation of $MeSn^{3+}$ by the carbanion donor. The combination of MeI and $Me_2Co(N_4)^+$ produced the greatest net yield of MeSn.

Other methyl donors. Hamasaki *et al.* (1991) tested several possible methylating agents: methanol, ethanol, acetic acid, propionic acid, hexamethyldisiloxane, humic acid, and fulvic acid. Among these, they found that only ethanol produced MeSn when incubated with inorganic tin. The lower the pH, the higher the amount of MeSn formed. At higher pH, tin hydroxides may form which are inert to methylation. Changing temperature had little effect on the overall yield of MeSn. Increasing the chloride ion concentration from 0 to 30 g L^{-1} significantly decreased the MeSn yield indicating a greater potential for methylation by ethanol in fresh rather than salt water. Their observations were consistant with a successive methylation scheme. If samples are irradiated (UV light, λ = 300 - 420 nm) methanol and propionic acid are also capable of methylating Sn(II) and, to a much lesser extent, Sn(IV). No MeSn were observed in the samples where inorganic tin was methylated with humic or fulvic acid. This does not conclusively indicate that these compounds are not capable of methylating inorganic tin because MeSn are adsorbed by humic and fulvic acids and may not be extracted by conventional methods.

Instrumentation

An abundance of instrumental methods exist that are capable of detecting total tin in environmental samples. Much of the tin present in marine systems may be in the form of the more toxic MeSn. For example, in fish MeSn make up 3 - 6% of the total tin, while in

limpets MeSn may comprise up to 75% of the total (Tugrul *et al.*, 1983). Therefore, it is important to be able to determine concentrations of individual MeSn species. Many of the speciation techniques that follow consist of 4 basic steps: derivatization to form volatile MeSn, trapping, separation, and detection. Each of these steps will be discussed individually with emphasis on the various possibilities that exist to accomplish them.

Derivatization of Methyltin Compounds

The goal of various derivatization methods has been to render the MeSn volatile. Many of the earlier researchers generated mixed methylbutyltin compounds, $Me_nBu_{4-n}Sn$, via reaction with the Grignard reagent, BuMgCl (Chau *et al.*, 1982, Forsyth and Cleroux, 1991). The derivitives formed have the advantage of being stable toward rearrangement and the method is less prone to matrix interferences than other derivitization methods (Szpunar-Lobinska *et al.*, 1995). However, Grignard synthesis is time-consuming and tedious. An *in situ* ethylation technique was developed by Rapsomanikis *et al.* (1986) for use in speciation of methyllead compounds. Addition of aqueous sodium tetraethylborate generates volatile methylethyllead compounds. This technique was adapted for speciation analysis with methylmercury compounds (Bloom and Effler, 1990) and, later, for use with inorganic tin compounds (Ashby and Craig, 1991b). This method has the advantage of being much simpler to use than the Grignard synthesis, however, of the organotin species analyzed, only MeSn form volatile ethyl derivatives. A simpler, on line technique used by many researchers

(Gilmour *et al.*, 1985; Braman and Tompkins, 1979; Anderae and Byrd, 1984) involves the generation of MeSn hydrides. This method requires the addition of dilute sodium borohydride ($NaBH_4$) (4 - 6%) to a slightly acidic (pH = 3 - 6.5) solution of MeSn. Martin and Donard (1995) found that hydride generation is slightly more sensitive than the ethylation technique, however, in complex matrices, it is more prone to interferences from organic compounds. Also, at high temperatures the hydride derivatives may rearrange (Chau, 1988). Owing mainly to its ease in handling, hydride generation was the derivatization method of choice for this research. Generally, the organotin compounds are extracted from the organic matrix and post digestion spikes demonstrate no inhibition of hydride generation. Additionally, separation and elution of MeSn hydrides occurs at ambient temperatures (≤50°C) and no rearrangment has been observed.

Some recent advances in the hydride generation step of this technique have included continuous, on-line, and on-column hydride generation. Le *et al.* (1992) developed a continuous hydride generator that combined hydride production and separation of gas and liquid phases in one unit. Their method utilizes a Buchner funnel with a fine porous frit within a glass cylinder with an overflow outlet. Samples run using their generator exhibit lower signal-to-noise and less susceptability to interferences. Krull and Panaro (1985) used on-line hydride generation followed by direct current plasma (DCP) emission spectroscopy (ES) for trace determination of total tin. The hydride generation step is post column, on line and real time. On line hydride generation has the advantage of being easily automated, however, unlike Le *et al.*'s technique, there is a loss of sensitivity by an order of magnitude.

Other problems encountered include differing reaction rates and blank signals. Clark *et al.*, 1987 developed an on-line hydride generation system involving a packed gas chromatographic (GC) column containing a $NaBH_4$ amended fraction in the top 5 cm. Solvent extract of samples can be directly injected onto the column where they are derivatized, separated and detected.

Trapping Techniques

Since many of the MeSn speciation techniques involve on-line derivitization, trapping is necessary to focus compounds at the head of the column. This serves to concentrate the analytes from the reaction mixture, often enhancing the sensitivity of the method by orders of magnitude. Additionally, the analytes are focussed in a narrow band at the head of the column prior to separation, minimizing band broadening as the analytes travel through the column, resulting in sharper, more reproducible peaks.

Gilmour *et al.* (1985) trapped methyltin hydrides in Tenax-GC packed columns and desorbed them by ballistic heating to 200°C. Jackson *et al.* (1982) used a commercial purge-trap device packed with Tenax-GC to introduce compounds to a GC-Flame Photometric Detection (FPD) system. This method is useful if the presence of water in the analytical system is a problem since water is not trapped by the Tenax. Another advantage to the Tenax trap is that MeSn species can be stored on it at room temperature for a period of time without decomposition and then desorbed to a separation and detection system.

Many researchers (Braman and Tompkins, 1979, Randall *et al.*, 1986a, Le *et al.*, 1992) cryogenically trap compounds at the head of the analytical column by immersing it in liquid nitrogen. MeSn are desorbed at room temperature or slightly above. A trapping system utilizing room temperature and below minimizes the possibility of redistribution reactions.

Separation

Separation of the volatile MeSn is possible by either high performance liquid chromatography (HPLC) or by GC. Krull and Panaro (1985) accomplished speciation by interfacing direct current plasma emission spectroscopy with HPLC. Ebdon *et al.* (1985) were able to differentiate between Sn(II), Sn(IV) and $(CH_3)_3Sn^+$ using HPLC with flame AAS. They interfaced the HPLC to the AAS with a discrete volume nebuliser. While HPLC is useful for compounds that do not form volatile derivatives without loss of identifying information (metalloamino acids, metalloproteins), it suffers from a lack of sensitive, element selective detectors.

Chromatographic separation is based on partitioning of the analytes between a stationary phase and a mobile phase. The amount of time a particular analyte spends in each phase is determined by a distribution coefficient, which must be different for each analyte in order for separation to occur. A somewhat different approach involves the use of both a carrier stream and a stationary phase which are relatively inhert to the analytes. If the

analytes are frozen at the head of the column, they may proceed through the column relatively unimpeded once they reach their boiling points. In this case, analytes are separated based on their different boiling points, rather than their affinity for a particular mobile or stationary phase as in a true chromatographic separation.

Many researchers (Gilmour *et al.*; 1985, Donard *et al.*; 1986, Jackson *et al.*, 1982) used columns packed with a liquid phase on a solid support combined with cryogenic trapping to separate MeSn. Liquid phases used were 3% SP-2401 with 10% SP-2100 (Jackson *et al.*, 1982; Gilmour *et al.*, 1985), 0.2% carbowax (Gilmour *et al.*, 1986), 15% OV-3 (Anderae and Byrd, 1984) and 10% SP-2100 (Rapsomanikis *et al.*, 1986). All of these materials enabled good separation of volatile MeSn.

More recently, there has been a trend toward the use of open-tubular megabore or capillary columns with polymethoxysilane coatings for separation of organometallic compounds (Szpunar-Lobinska, 1995). These often provide greater resolution of organometallic species and sharper, more concentrated bands. Additionally, open-tubular columns may be operated at lower gas flow rates, increasing the residence time of the analyte in the detector and, ultimately, increasing the sensitivity. However, in capillary columns the sensitivity advantage is often lost because of their restricted capacity.

Burns *et al.* (1987) compared a GC equipped with a Katharometer detector (KD) and HPLC with a differential refractrometer for speciation of MeSn. The 2 m gas-liquid column

packed with silicone rubber SE-30 was incapable of separating $MeSnCl_3$ and Me_2SnCl_2. At the operating temperature (90°C) redistribution reactions occurred.

$$Me_4Sn + MeSnCl_3 \rightarrow Me_3SnCl + Me_2SnCl_2 \qquad \text{(Equation 1.9)}$$

Therefore, all 4 MeSn could not be detected in the same mixture. Absolute detection limits for GC-KD were 8.25 μg. Using HPLC with a reverse phase column provided good separation of all four MeSn, but there was a decrease in sensitivity (LOD = 80 - 100 μg).

Detection

Detection systems must be compatible with the separation system and sensitive enough to detect environmental concentrations of MeSn (ng g^{-1}). Several detectors have been used that fit these requirements. Most commonly used are emission spectroscopy (ES), atomic absorption spectroscopy (AAS), and mass spectrometry (MS).

The sensitivity of atomic emission techniques relies heavily on the excitation source since the greater the excitation efficiency, the more intense the emission. Braman and Tompkins (1979) and Andreae and Byrd (1984) utilized hydrogen rich H_2-air flame emission. Limits of detection for both methods were in the pg range with a linear range from 0.1 - 5 ng. Method precision averaged about 5% over the linear range of the response curve. Problems

encountered include interference from emission bands of organic compounds. The advent of plasma emission sources greatly enhanced the viability of emission detectors for environmental samples. The sensitivity of emission techniques is at the pg level when coupled to a plasma source (Chau and Wong, 1991). Krull and Panaro (1985) used direct current plasma emission spectroscopy (DCP-ES) to detect alkyltin compounds separated by HPLC. Detection limits were *ca.* 25 pg ml^{-1}. More recently, microwave induced plasma has been coupled with AES for multi-element detection of mercury, lead, and tin compounds separated by gas chromatography (Minganti, *et al.*, 1995). This is one of the most sensitive methods reported to date, with a detection limit of 1 pg.

Several investigators have taken advantage of the sensitivity and specificity of atomic absorption detectors. There are 3 methods of atomization that have been used extensively; flame, graphite furnace, and quartz furnace. Burns *et al.* (1981) compared flame atomization to electrothermal atomization in a quartz furnace and found that methods utilizing the quartz furnace were generally more sensitive (LOD = 2 pg as Sn), but that flame atomization was useful for liquid eluent from HPLC. Andreae and Byrd (1984) investigated the possibilities of using either graphite furnace or quartz furnace AAS. Both systems used an electrodeless discharge lamp operated at 224.7 nm as the source. The noise limited detection limit for the graphite furnace method was 50 pg with a linear range from 50 pg to 50 ng. Use of the quartz furnace also enabled a detection limit of 50 pg, however, there was interference from spurious peaks eluting along with each MeSn peak. Other researchers have not noted these spurious peaks. Chau *et al.* (1982) detected MeSn by AAS in a quartz furnace interfaced with

GC. Standards gave reproducible results (coefficient of variation, 5-7 % for the 3 MeSn), good sensitivity (LOD = 0.1 ng as Sn), and a working range of 0.1 - 33 ng as inorganic tin. This system is also capable of detecting Me_4Sn and volatile MeSn hydrides, which may be present in tin contaminated harbors. Forsyth and Hayward (1995) improved both sensitivity and precision in the quartz furnace technique by redesigning the quartz furnace. Use of a smaller diameter (7 mm i.d.) quartz T-tube encased in a ceramic fiber insulated furnace unit enabled detection limits for butyltin compounds in the low pg range and response reproducibiltity ranging from 1.8 to 3.9%.

Gilmour *et al.* (1985, 1986) speciated MeSn by MS operated in electron impact mode with selective ion monitoring (SIM). All three MeSn gave linear response curves from 0.1 - 15 ng. Detection limits were between 3 and 5 pg as Sn. Lawson and Ostah (1993) used tandem mass spectrometry for speciation of organotin compounds without prior separation or derivatization. The samples are loaded in the capillary probe tip, which is placed in the MS and heated from ambient temperature to 400°C at 230°C per min. The resulting fragmentation pattern is sufficient to enable identification of individual organotin compounds in a simple mixture.

According to Chau and Wong (1991) of all detection systems coupled to chromatographic separation instruments, AAS is the most sensitive, specific, and widely used for metal and organometal speciation. In light of all previous research, we used the MeSn

speciation method developed by Donard *et al.* (1986) for most of our work. It involves volatilization of compounds from aqueous medium by hydride generation, separation in a 'U' tube packed with SP-2100 on a chromatographic support material, and detection by AAS in an electrothermally heated quartz furnace with a hydrogen rich oxygen-hydrogen flame. It attains absolute detection limits of 20-50 pg as inorganic tin with a linear range up to 30 ng.

The advantage of using mass spectrometric detection for MeSn is that it allows for a second axis of separation. The MS inherently separates all compounds based on mass of the ion fragments produced. The benefit provided is positive identification of MeSn in the sample and it allows for isotopic determination. In the final experiment of this disertation (Chapter 4), a Hewlett-Packard Gas Chromatographic Mass Spectrometer was used for the separation and detection steps, respectively, in the speciation scheme.

Summary

This study attempts to shed some light on the complexities of biogeochemical cycling of tin in the estuarine environment. The two separate seasonal studies reported have only emphasized how complex the situation is. For example, a study conducted in 1989 (Weber *et al.*, 1991) demonstrated MeSn concentrations rose sharply at the beginning of the growing season (May 23) to a high of 470 ng g^{-1} then gradually decreased to *ca.* 10 ng g^{-1} on July 4, and remained at that level for the remainder of the growing season. Conversely, during a similar study conducted for the 1991 growing season (Falke and Weber, 1993), the MeSn concentrations ranged from 1.2 ng g^{-1} to 31 ng g^{-1}, with no apparent temporal trend. Decaying

S. alterniflora is likely to have an effect on the overall methylation of tin compounds in the estuary as there was statistically significant rearrangement of Me_2Sn^{2+} to Me_3Sn^{+} during a model decay study (Falke and Weber, 1994). In order to obtain more information from future environmental studies on MeSn, significant advances have been made on a gas chromatographic mass spectrometry technique for tin speciation. This will enable researchers to trace isotopically labeled tin through model and real systems. Additionally, it will provide a means of positive identification of environmental tin compounds.

CHAPTER II

SEASONAL METHYLTIN AND (3-DIMETHYLSULPHONIO)PROPIONATE CONCENTRATIONS IN LEAF TISSUE OF *Spartina alterniflora* OF THE GREAT BAY ESTUARY (NEW HAMPSHIRE)

Introduction

A potential source of metyltin compounds (MeSn) is methylation of inorganic tin by (3-dimethylsulfonio)propionate (DMSP). Estuarine cord grass, *Spartina alterniflora*, contains MeSn (Weber and Alberts, 1990) as well as DMSP (Dacey *et al.*, 1987). For this reason *S. alterniflora* leaves and associated sediments and rhizospheres may be significant sources of MeSn to the estuarine environment. In a laboratory experiment, Weber and Alberts (1990) found *ca.* 11 ng g^{-1} fresh weight $MeSn^{3+}$ and Me_2Sn^{2+} in *S. alterniflora* from Sapelo Island, GA after 5 days of hydroponic incubation in tin-amended solutions. Methylation of inorganic tin and demethylation of MeSn could occur within the rhizosphere of *S. alterniflora* or in association with the aerial plant, and MeSn could then enter the water column as the plants undergo senescence and decompose.

This study demonstrates that MeSn occur in *S. alterniflora* leaves throughout the growing season at three sampling sites in the Great Bay Estuary (NH) including Chapman's Marsh where Hines *et al.* (1989) studied the biogeochemistry of sulfate reduction. During the

time frame studied total MeSn concentrations in *S. alterniflora* increased to their seasonal high values early in the growing season in May, decreased within three weeks, and remained low until the last sampling date in September. The date of sampling, but not the site, was a significant factor in the variation in MeSn concentrations. These results are helpful in understanding the biogeochemical cycling of tin, and the sources and quantities of MeSn in the estuary. This knowledge could allow for assessment of potential hazards associated with MeSn in the estuarine environment.

It should be noted here that this work was initiated by Marilyn Billings who established the sampling sites, collected samples and performed all DMSP analysis. This work was published by Weber *et al.* (1991).

Methods

Materials

All fresh water used was doubly deionized and distilled through a Corning Mega-pure still. $MeSn^{3+}$, Me_2Sn^{2+}, Me_3Sn^{+}, and Sn(IV) chlorides (97+ % purity) were obtained from Alfa Chemicals. Stock solutions of *ca.*. 1000 $\mu g\ ml^{-1}$ (all concentrations are as Sn) were prepared in 1 M HCl. Standards were made by diluting stock solutions to *ca.*. 5 $mg\ ml^{-1}$ in 0.05 M HNO_3 (MeSn chlorides) or 1 M HCl ($SnCl_4$). Sodium borohydride ($NaBH_4$)

solution (6% w/v) was prepared by dissolving 12 g $NaBH_4$ (Aldrich Chemicals, 98%) in 100 ml water and allowing to stand, refrigerated, overnight. The solution was then filtered through a 0.2 μm polycarbonate filter (Nuclepore) to remove any inorganic tin colloids, and diluted to 200 ml with water. All other chemicals were of reagent grade. All glassware and plasticware were soaked 48 h in a 10% HNO_3 solution.

Sampling and Dry Weight/Fresh Weight Ratios

S. alterniflora samples were obtained weekly from May 8 to September 18, 1989 from three widely separated sites of the Great Bay Estuary: Johnson Creek, Crommett Creek, and Chapman's Marsh (Figure 2.1). Usually nine leaf samples were taken from each site on each date, measured, and weighed. When the leaves were more than approximately 90 cm long (June 27), 15 cm each were taken from tips, central part, and base. Dry weight/fresh weight ratios were determined immediately by placing two to three plants (above ground portion, only) in an oven for one week at 60° C. The average dry weight/fresh weight value for 129 plants was 0.272 ± 0.065. Other samples were stored in a freezer until MeSn and DMSP determinations were performed.

S. alterniflora leaf samples were removed from the freezer, pressed lightly between two sheets of Kaydry wipers (Kimberly-Clark), and weighed. Leaves were then ground to a fine powder in liquid nitrogen. Ground samples were transferred to a centrifuge tube with a

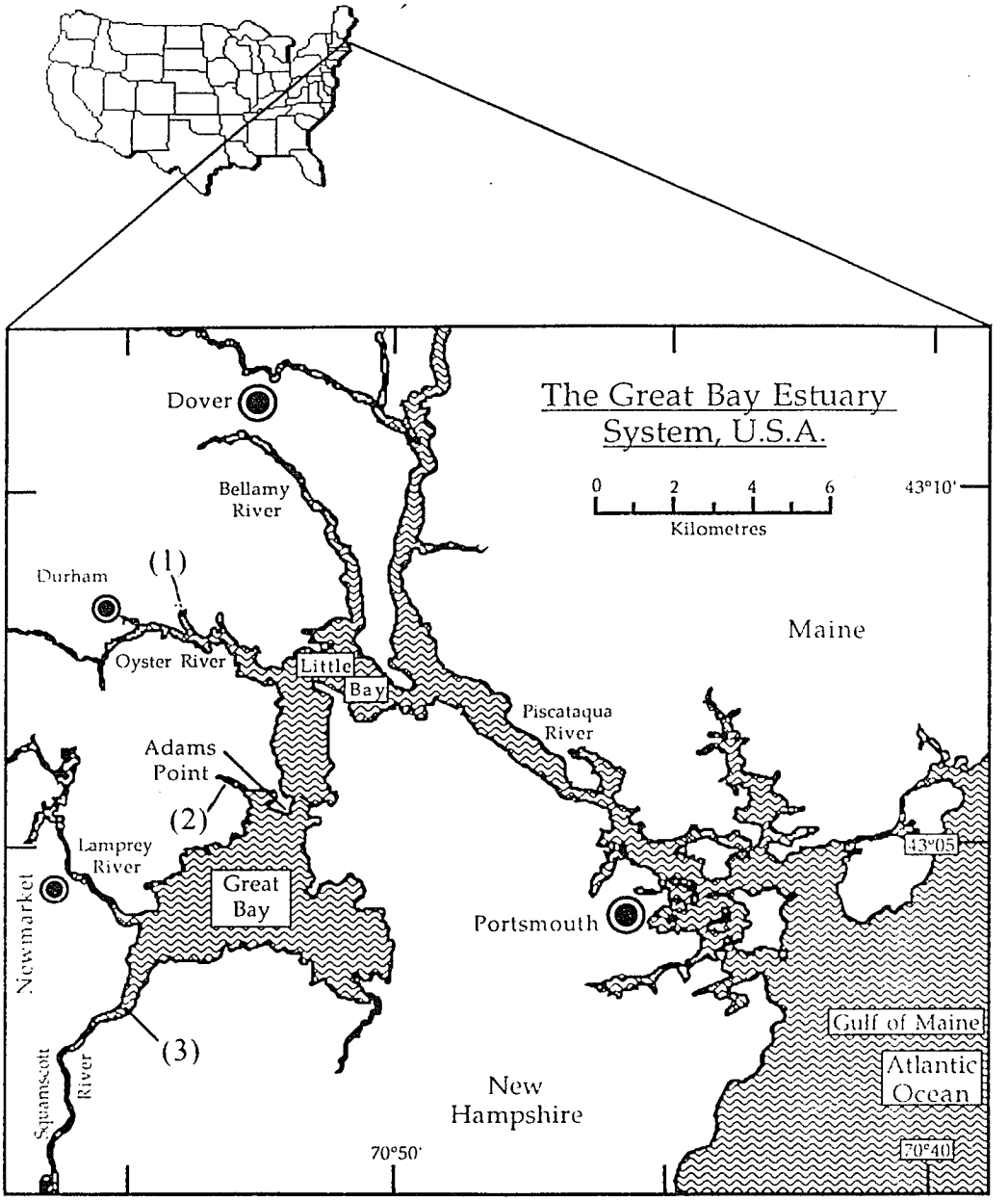

Figure 2.1. Sampling locations in the Great Bay Estuary: (1) Johnson Creek, (2) Crommett Creek, and (3) Chapman's Marsh.

spatula, extracted by sonication at 50°C for 1 h in 5 ml of 1 M HCl per 1 g fresh weight, and centrifuged (7500 rpm) for 15 min at room temperature. The supernatant liquid was removed, placed in acid-washed 20 ml glass scintillation vials, and frozen until MeSn concentrations were determined. Recoveries by this method using 40 ng ml^{-1} spikes were: $MeSn^{3+}$ (28 ± 3.7%), Me_2Sn^{2+} (94 ± 2.3%), and Me_3Sn^{+} (99 ± 8.4%).

Determination of Inorganic Tin and Methyltin Compounds

Inorganic tin and MeSn in each sample were determined as tin atoms by on-line hydride generation followed by cyrogenic trapping, chromatographic separation and detection by atomic absorption spectrometry (Figure 2.2) (Francois and Weber, 1988). Samples of 0.1 to 2 ml were placed in the hydride generation flask with 40 ml H_2O and acidified through the addition of 0.5 ml acetic acid. Acidic extracts were first neutralized through equal molar additions of 10 M KOH. After the flask was connected to the column, 2.5 ml of $NaBH_4$ solution was added. This has the dual purpose of generating the hydrides of the tin compounds and creating a large volume of hydrogen gas which serves to purge Sn(IV) hydride and MeSn hydrides from the flask.

All hydrides were trapped in a liquid nitrogen cooled 'U' tube (40 cm x 5 mm i.d.) packed with 3% SP-2100 on Chromosorb G AW-DMCS 45/60 which has been silanized with dimethylsilanylchloride (5% (v/v) in toluene) (Francois and Weber, 1988). Prepurified

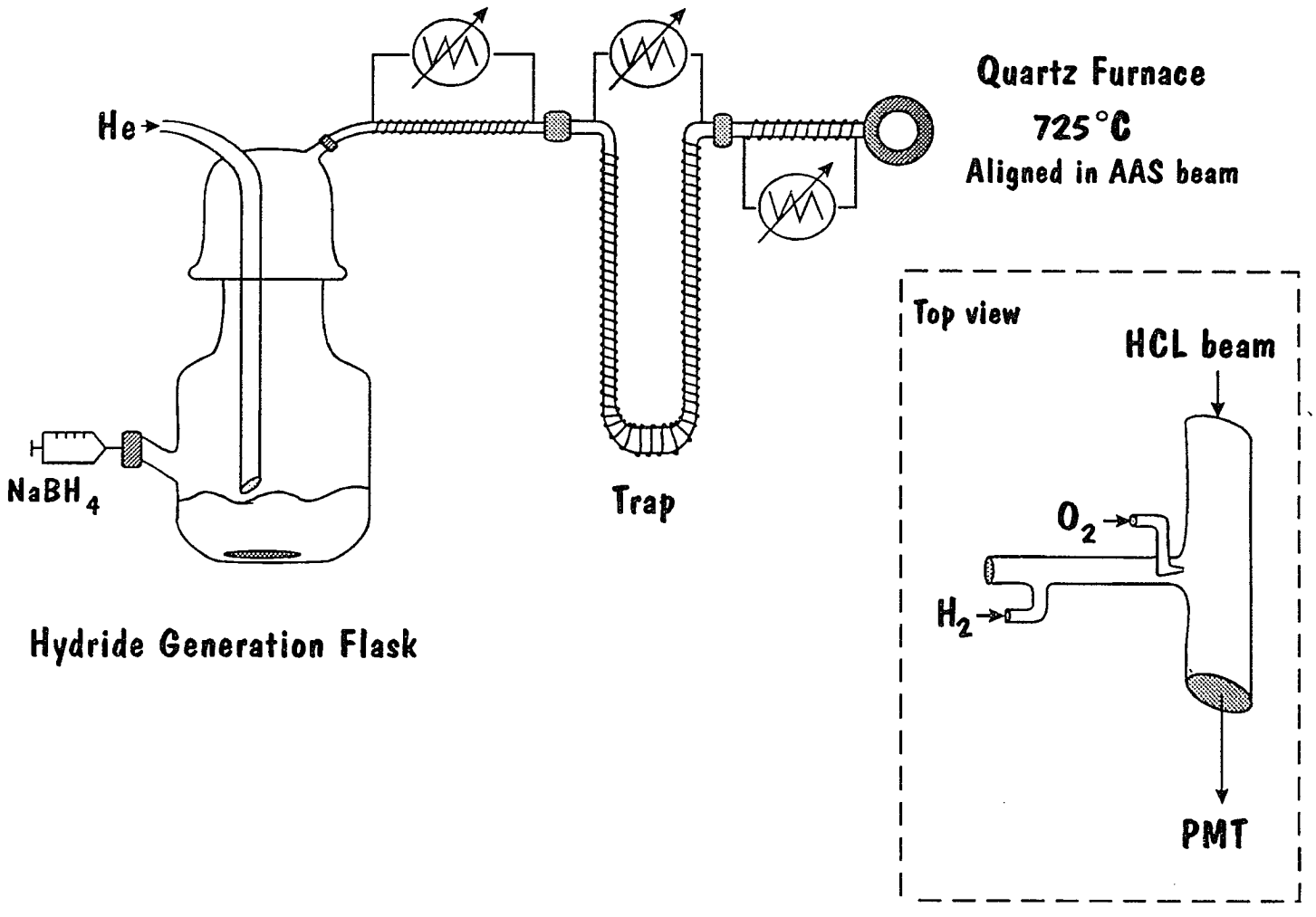

Figure 2.2. Schemata of hydride generation-atomic absorption spectrometer used for inorganic tin and methyltin speciation.

helium at a flow rate of 120 ml min^{-1} was the carrier gas. Upon removal of liquid nitrogen from the trap, tin compounds eluted according to their boiling points and were atomized in an electrothermally heated (750°C) quartz furnace. A hydrogen-rich oxygen-hydrogen flame (H_2 flow rate = 830 ml min^{-1}, O_2 flow rate = 21 ml min^{-1}) improved the atomization efficiency of the hydrides. Tin atoms were detected using a tin hollow cathode lamp (λ = 286.3 nm) in a Perkin Elmer 503 Atomic Absorption Spectrophotometer. Retention times are 0.7, 1.5, 2.1 and 2.7 min for SnH_4, $MeSnH_3$, Me_2SnH_2, Me_3SnH respectively. Absolute limits of detection (3 times the standard deviation of baseline noise) are 0.25 ng as Sn for each of the tin compounds. Actual detection limits depend on dilution factors and volume of aliquot used. Quantitation is based on calibration curves of the analytes. A midpoint standard was analyzed after every 10 analysis to verify instrument response. Each sample determination was performed at least in duplicate. If the relative percent deviation is greater than 10%, a third sample aliquot was analyzed.

Results

Inorganic Tin and Methyltin Concentrations

Inorganic tin, $MeSn^{3+}$, and Me_2Sn^{2+} were found consistantly at all 3 sampling sites (Table 2.1). Additionally, but not shown in Table 2.1, we found 3 to 5 ng g^{-1} of Me_3Sn^+ in *S.*

Table 2.1. Concentrations (ng Sn g^{-1}) ± 1 standard deviation of total recoverable inorganic tin (Sn), monomethyltin ($MeSn^{3+}$), and dimethyltin (Me_2Sn^{2+}) in leaf tissue of *S. alterniflora* during the 1989 growing season.

Date	Compound	Johnson Creek[a]	Crommett Creek[a]	Chapman's Marsh[a]	**Mean[b]**
May 8	Sn	15.0 ± 5.0	11.5 ± 6.0	45.8 ± 7.6	**24.1 ± 10.9**
	$MeSn^{3+}$	5.7 ± 2.2	3.6 ± 1.7	8.5 ± 5.9	**6.0 ± 6.5**
	Me_2Sn^{2+}	1.0 ± 0.5	5.7 ± 4.9	2.0 ± 0.7	**2.9 ± 5.0**
May 15	Sn	12.6	45.1	18.6	**25.4 ± 17.3**
	$MeSn^{3+}$	13.1	192	38.6	**81.2 ± 96.8**
	Me_2Sn^{2+}	3.1	40.6	5.6	**16.4 ± 21.0**
May 23	Sn	69.4 ± 51.3	23.3 ± 15.5	39.2 ± 12.8	**44.0 ± 55.1**
	$MeSn^{3+}$	322 ± 85	168 ± 35	448 ± 363	**312 ± 374**
	Me_2Sn^{2+}	195 ± 69	144 ± 51	140 ± 55	**160 ± 102**
May 29	Sn	30.3 ± 31.0	20.9 ± 13.6	24.5 ± 0.8	**25.2 ± 33.9**
	$MeSn^{3+}$	62.0 ± 31.8	122 ± 88	145 ± 38	**110 ± 101**
	Me_2Sn^{2+}	58.2 ± 22.1	152 ± 56	128 ± 26	**113 ± 66**
June 7	Sn	5.4 ± 3.0	16.2 ± 2.6	7.5 ± 4.9	**9.7 ± 6.3**
	$MeSn^{3+}$	17.6 ± 6.4	37.4 ± 4.6	19.8 ± 3.5	**24.9 ± 8.6**
	Me_2Sn^{2+}	17.5 ± 1.2	45.2 ± 9.8	19.1 ± 6.0	**27.3 ± 11.6**
June 13	Sn	9.0 ± 0.7	19.9 ± 11.4	4.5 ± 4.4	**11.1 ± 12.2**
	$MeSn^{3+}$	49.1 ± 8.7	13.8 ± 0.9	17.8 ± 1.4	**26.9 ± 8.9**
	Me_2Sn^{2+}	31.2 ± 4.2	10.2 ± 5.2	23.2 ± 1.2	**21.5 ± 6.8**
June 20	Sn	7.8 ± 3.3	7.6 ± 1.4	4.8 ± 5.3	**6.8 ± 6.4**
	$MeSn^{3+}$	7.6 ± 4.6	7.4 ± 3.8	8.0 ± 3.3	**7.7 ± 6.8**
	Me_2Sn^{2+}	7.7 ± 6.2	6.5 ± 3.5	9.7 ± 8.6	**8.0 ± 11.2**
July 4	Sn	32.6 ± 40.1	9.4 ± 4.9	8.8 ± 1.5	**16.9 ± 40.4**
	$MeSn^{3+}$	7.2 ± 3.9	7.2 ± 2.0	7.0 ± 2.3	**7.2 ± 4.9**
	Me_2Sn^{2+}	4.4 ± 2.4	3.2 ± 0.9	1.9 ± 0.7	**3.2 ± 2.7**
July 19	Sn	44.8 ± 31.3	18.9 ± 23.5	6.4 ± 5.5	**23.4 ± 39.5**
	$MeSn^{3+}$	3.9 ± 0.9	3.3 ± 1.3	3.0 ± 0.4	**3.4 ± 1.6**
	Me_2Sn^{2+}	3.7 ± 1.5	2.3 ± 0.8	1.4 ± 0.3	**2.5 ± 1.7**

Table 2.1. (Continued)

Date	Compound	Johnson Creek	Crommett Creek	Chapman's Marsh	**Mean**
August 1	Sn	43.7 ± 35.7	28.4 ± 17.8	6.2 ± 1.5	**26.1 ± 39.9**
	$MeSn^{3+}$	2.7 ± 0.5	4.1 ± 1.8	4.2 ± 1.0	**3.7 ± 2.1**
	Me_2Sn^{2+}	3.6 ± 1.8	2.8 ± 0.7	1.8 ± 0.7	**2.7 ± 2.1**
August 16	Sn	19.7 ± 4.6	66.0 ± 39.8	25.2 ± 8.1	**39.6 ± 40.9**
	$MeSn^{3+}$	9.2 ± 2.8	9.3 ± 3.9	7.1 ± 4.1	**8.6 ± 6.3**
	Me_2Sn^{2+}	2.1 ± 0.2	2.7 ± 0.6	1.7 ± 1.2	**2.2 ± 1.4**
August 29	Sn	9.9 ± 5.8	187 ± 17	6.8 ± 1.3	**68.0 ± 18.0**
	$MeSn^{3+}$	4.0 ± 1.1	3.7 ± 0.2	5.0 ± 1.3	**4.3 ± 1.7**
	Me_2Sn^{2+}	2.1 ± 0.4	5.7 ± 2.2	2.2 ± 0.4	**3.3 ± 2.3**
September 18	Sn	8.7 ± 1.5	11.7 ± 2.5	4.3 ± 2.4	**8.2 ± 3.8**
	$MeSn^{3+}$	3.3 ± 1.5	4.9 ± 1.9	4.2 ± 0.6	**4.2 ± 2.5**
	Me_2Sn^{2+}	1.7 ± 0.7	2.3 ± 0.6	2.4 ± 0.5	**2.1 ± 1.0**

[a]Standard deviation represents deviation in analysis only.
[b]Standard deviation represents deviation resulting from multiple sites and multple analysis at each site.

alterniflora leaf tissue at one or two sites on June 13, July 4, July 19, and August 16. Because of the small amount of data for Me_3Sn^+, we will not discuss it further. On July 4 and August 1, an organotin compound with a retention time similar to that of monobutyltin hydride occurred at two of three sites. No attempt was made to determine the identity of the compound.

Analysis of variance (ANOVA) (see Appendix for description) calculations of inorganic tin, $MeSn^{3+}$, or Me_2Sn^{2+} concentration *vs.* sampling site by date showed that the site was a significant contributor to the variability ($P \leq 0.05$) in only 8 of 36 (12 dates and 3 compounds) sample sets. Thus, it seemed reasonable to average data from the three sites. Two-way ANOVA calculations of inorganic tin, $MeSn^{3+}$, or Me_2Sn^{2+} concentrations *vs.* date and site (Table 2.2) supported this contention by showing that the concentration did not vary significantly by site ($P = 0.3$ to 0.7). The date was also an insignificant source of variation for inorganic tin data ($P = 0.6$), but is a very significant factor ($P = 0.00$) for $MeSn^{3+}$ and Me_2Sn^{2+} concentrations. ANOVA analysis strongly supported the validity of using averages of the three sites in the following discussion.

Average $MeSn^{3+}$ and Me_2Sn^{2+} concentrations (Figure 2.3) for all three sampling sites showed a very strong trend of decreasing concentrations from spring to autumn. For example, average $MeSn^{3+}$ concentrations over all three sites decreased from 312 ng g^{-1} on May 23 to 26.9 ng g^{-1} on June 13 and remained between 3 and 9 ng g^{-1} until September 18. Me_2Sn^{2+}

Table 2.2. Distribution of variability (by percent) of (3-dimethylsulfonio)propionate (DMSP), inorganic tin (Sn), and methyltin concentrations among date, site and residual (random error)[a]. A large portion of the variability attributed to a particular factor indicates a significant effect of that factor on any changes in the concentration. The probability of the factor having no effect on the total variance is included in parenthesis.

Compound	Date (%)	Site (%)	Residual %
DMSP	4.5 (0.55)	90.6 (0.00)	5.0
Sn	26.7 (0.60)	41.8 (0.28)	31.5
$MeSn^{3+}$	86.9 (0.00)	3.3 (0.71)	9.8
Me_2Sn^{2+}	93.3 (0.00)	2.6 (0.55)	4.1

[a]Analysis of Variance results, STATA statistics package.

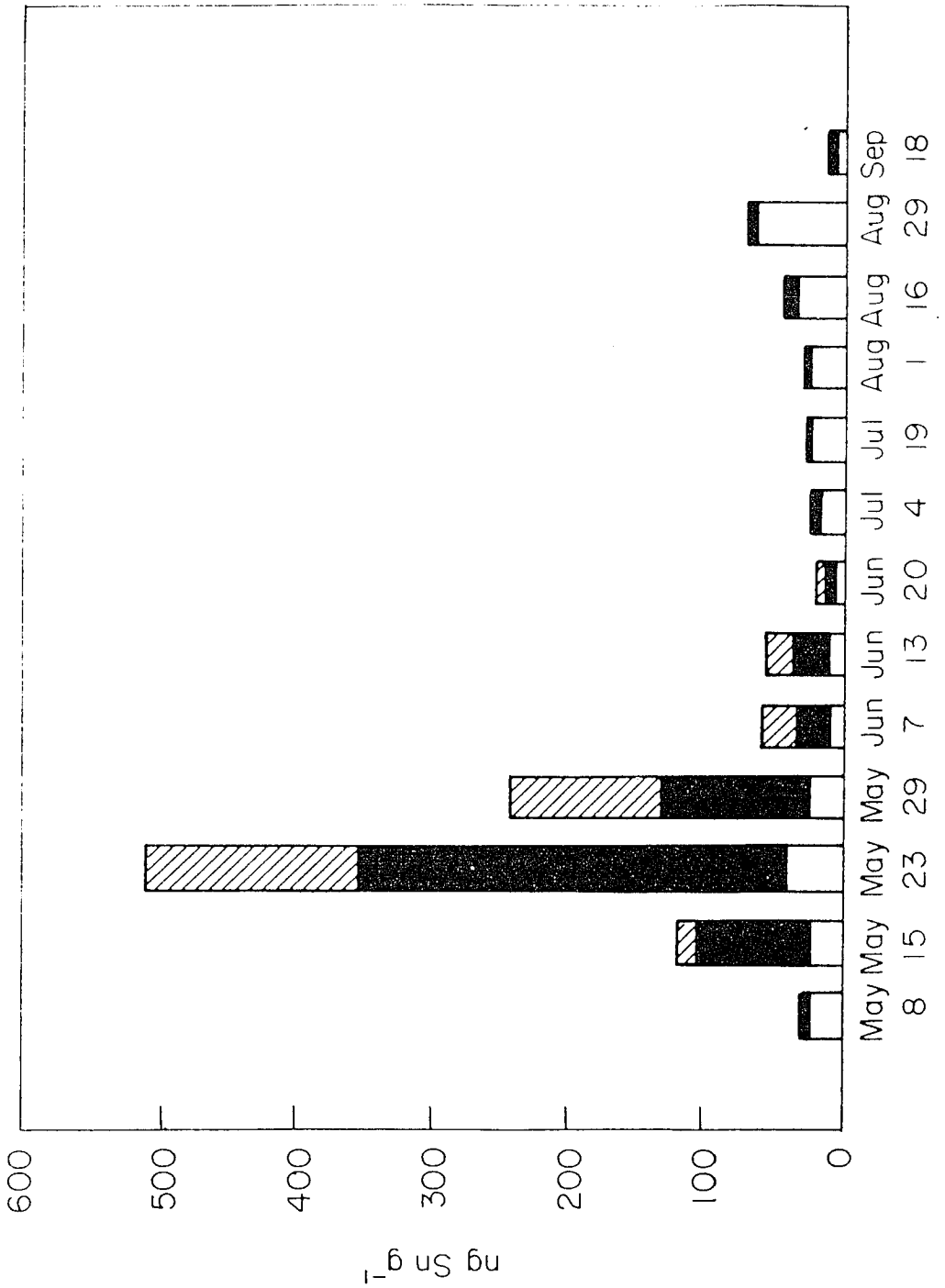

Figure 2.3. Concentrations of inorganic tin (□), $MeSn^{3+}$ (■), and Me_2Sn^{2+} (▨) in leaf tissue of *S. alterniflora* during the 1989 growing season.

concentrations, but not inorganic tin concentrations, showed a similar periodic trend. Inorganic tin concentrations followed a sinusoidal pattern with a 3 month period.

Discussion

Fundamental concepts of methyl transfer reaction mechanisms (Rapsomanikis and Weber, 1986) are essential to understanding the potential relationship between DMSP and MeSn concentrations in *S. alterniflora*. DMSP is a potential carbocation (Me^+) donor to Sn(II). Me^+ donors typically transfer methyl groups to oxidizable methyl acceptors such as Sn(II) (Equation 2.1).

$$Sn(II) + Me^+ \rightarrow MeSn(IV)^{3+} \qquad \text{(Equation 2.1)}$$

Sn(IV) is in its highest oxidation state, and cannot react with Me^+ because that process represents a formal 2-electron oxidation of the acceptor. Further methylation to Me_2Sn^{2+} or Me_3Sn^+ is difficult to explain because methylation of $MeSn^{3+}$ would require preceding reduction of methyltin(IV) compounds to their unknown methyltin(II) analogues. Me_2Sn^{2+} and Me_3Sn^+ compounds alternatively can form via rearrangement processes (Equations 2.2 and 2.3).

$$2\ MeSn(IV)^{3+} \rightarrow Me_2Sn(IV)^{2+} + Sn(IV)^{4+} \qquad \text{(Equation 2.2)}$$

$$2\ Me_2Sn(IV)^{2+} \rightarrow Me_3Sn(IV)^{+} + MeSn(IV)^{3+} \qquad \text{(Equation 2.3)}$$

Methylation of Sn(II) to $MeSn^{3+}$ (Equation 2.1) by methyl iodide, a Me^{+} donor, readily occurs in model estuarine systems (Rapsomanikis and Weber, 1985; Lee and Weber, 1988; Ring and Weber, 1988). Ring and Weber (1988) attempted in similar experiments to methylate Sn(II) with DMSP in a chemical process, but the reaction did not occur. However, in an enzymatic process, DMSP could methylate Sn(II) resulting in MeSn in *S. alterniflora* leaf tissue in at least two ways. The reaction could occur in pore water with MeSn absorbed by the plant or methylation could occur in or on *S. alterniflora* leaves.

In leaf samples taken on all dates except May 29, June 7, and June 20, the average $MeSn^{3+}$ concentration in *S. alterniflora* was greater than the average Me_2Sn^{2+} concentration; and Me_3Sn^{+} was rare. These observations suggest that methylation of inorganic tin to $MeSn^{3+}$ (Equation 2.1) is the predominant process. Less common successive methylation processes or rearrangement processes (Equations 2.2 and 2.3) could account for the observed Me_2Sn^{2+} and Me_3Sn^{+}. In agreement with these results, $MeSn^{3+}$ concentrations are generally higher than Me_2Sn^{2+} in water and sediments (Weber *et al.*, 1988), shellfish (Han and Weber, 1988), macroalgae (Donard *et al.*, 1987), and eelgrass (Francois and Weber, 1988; Francois *et al.*, 1989) of the Great Bay Estuary (NH).

Correlation of DMSP and $MeSn^{3+}$ concentrations by date and site resulted in a slope of -0.002. The probability that any correlation is a result of random error is 85%. This seems to indicate that there is no causal effect of DMSP on methylation of inorganic tin. This conjecture is reasonable assuming constant inorganic tin concentration. If DMSP were involved in methylation, constant DMSP concentration would result in constant MeSn production in a kinetics model. As this does not occur, it is unlikely that DMSP is involved in the methylation of inorganic tin.

One possible source of MeSn in leaf tissue is methylation of tin by sulfate reducing bacteria (SRB) in sediments (Gilmour *et al.*, 1987) followed by MeSn uptake from sediments into leaves. This explanation does not agree with the observations of Hines *et al.* (1989) who studied sulfate reduction at Chapman's Marsh during the 1984 and 1985 growing seasons. They observed that the maximum rate of sulfate reduction occurred during the summer growing season when we found decreasing MeSn concentrations (Table 2.1, Figure 2.3). Because of the inverse relationship between SRB activity (in previous years) and MeSn concentration, it is difficult to attribute MeSn in leaves to methylation of tin in sediments by SRB during the growing season. However, the source of MeSn could be slow SRB activity during cold months. Accumulated MeSn could then quickly translocate from sediment or rhizomes to leaf tissue during the early growing season.

A more plausible explanation for seasonal changes in MeSn concentrations is comparable to the relationship between carbohydrate production and the growing season for

S. alterniflora. Lytle and Hull (1980) observed that concentrations of carbohydrates stored in rhizomes decrease early in the growing season and increase during the peak growing season. Thus, when plants are entirely below ground or very short, carbohydrates move from rhizomes to leaf tissue; during the growing season carbohydrate movement is back toward the rhizomes. Because MeSn concentration was high in our study only during the early growing season, it is possible that MeSn also move to the leaves during the spring. Then MeSn are diluted as the plant grows during the summer and their concentrations decrease. Alternatively, after the early growth period, MeSn are formed and decomposed (or removed) in the leaves at approximately the same rate.

Rapid changes in MeSn concentrations in leaf tissue is undoubtedly related to complex processes involving the biogeochemistry of plants and sediments. Added biomass during the summer is unlikely to explain all changes in MeSn concentrations during the growing season. We attempted to confirm that dilution of MeSn assimilated in the spring during the growing season is the major cause of the observed trend by using leaf mass data collected at each site on each date. We normalized all MeSn concentrations by multiplying them by plant mass (above ground portion). Ideally, if MeSn concentrations decrease by dilution as leaf mass increases, the normalized values should not vary significantly by date in ANOVA calculations. However, ANOVA calculations on normalized $MeSn^{3+}$ against sites and dates gave the same result as measured concentrations-site is insignificant and date is significant in contribution toward variability. However, the percent of variance due to date decreased from 87% for $MeSn^{3+}$ concentrations (Table 2.2) to 74% for normalized values.

Apparently increasing biomass is only one of the factors that explains decreasing MeSn concentrations during the growing season. MeSn may also be further methylated to volatile $(CH_3)_4Sn$ and lost via transpiration through leaf stomata.

Measurement of MeSn in *S. alterniflora* leaf tissue alone cannot lead to an understanding of complex processes involving MeSn in salt marshes. A future study (Chapter III) will include determinations of MeSn below ground: In sediments, roots, rhizomes, and pore water. This should give us a clearer idea of whether MeSn are translocated from roots/rhizomes to leaf tissue during the early growing season.

CHAPTER III

VARIATIONS IN CONCENTRATIONS OF METHYLTIN COMPOUNDS AND INORGANIC TIN IN *Spartina alterniflora* AND POREWATER IN THE GREAT BAY ESTUARY (NEW HAMPSHIRE) DURING THE 1991 GROWING SEASON

Introduction

In a previous study (Weber *et al.*, 1991), we discovered a maximum in the total methyltin (MeSn) concentration in late May which decreased to approximately 5 ng g^{-1} in July and August. The purpose of the study reported here was to continue this investigation and determine concentrations of MeSn in leaves, roots, and rhizomes of *S. alterniflora*, as well as surrounding porewater, during a growing season. It was hoped this would give us a clearer idea of whether MeSn are translocated from the roots and rhizomes to the leaves early in the growing season and then distributed throughout the leaves as they grow. In order to quantitatively determine MeSn concentrations, we improved our previous extraction technique for leaves (Weber *et al.*, 1991) and developed adequate extractions for roots, rhizomes, and porewater.

Sample Extraction

A critical step in analyzing any biological sample for a trace constituent is quantitative extraction from the sample matrix. When performing speciation analysis, this problem is exacerbated. Not only is complete analyte extraction important, but, in order to have meaningful data, it is crucial that the extraction process not alter the species. For kour study, the extraction needed to be vigorous enough to remove MeSn from a fiberous cordgrass, but not so strong that carbon-tin bonds would be destroyed or that any transmutation of species would possibly occur. Another problem that had to be considered in the final solution was that in many samples the concentration of inorganic tin was significantly greater than that of MeSn. A large amount (> *ca.* 30 ng) of inorganic tin obscures a much smaller monomethyltin ($MeSn^{3+}$) signal. Therefore, the ideal extractant would remove all of the MeSn and very little of the inorganic tin without altering the identity of any of the species.

Previous experiments (Weber *et al.*, 1991) used 1 M HCl to extract MeSn from *S. alterniflora* leaves. Recoveries of 40 ng ml^{-1} MeSn spikes extracted by this procedure were 28 ± 3.7% of the $MeSn^{3+}$ spike, 94 ± 2.3% of the dimethyltin (Me_2Sn^{2+}) spike, and 99 ± 8.4% of the trimethyltin (Me_3Sn^{+}) spike. Based on these results, Me_2Sn^{2+} and Me_3Sn^{+} were effectively removed from the plant matrix with 1 M HCl, however $MeSn^{3+}$ was not. Use of 1 M HCl in 75% methanol (v/v) enables better recovery of $MeSn^{3+}$ (70%) from leaf material

while maintaining adequate extraction efficiencies for both Me_2Sn^{2+} (84%) and Me_3Sn^+ (87%).

Contrary to our previous study (Weber *et al.*, 1991) (Chapter II), no temporal trend in monomethyltin or inorganic tin concentrations was observed, but concentrations of these compounds did vary with plant part and porewater. This work was published by Falke and Weber (1993).

Experimental

Materials

Water used in all experiments was deionized two times and then distilled through a Corning Mega-pure still. Organotin standards are of 97+% purity from Alfa Chemicals. Stock solutions (ca. 1000 $\mu g\ ml^{-1}$) (all concentrations are as Sn) of methyltin chlorides and $SnCl_4$ were prepared in 1 M HCl, and diethyltin chloride was prepared in methanol. Standards were made by diluting stock solutions to ca. 5 $\mu g\ ml^{-1}$ in 0.05 M HNO_3 (MeSn chlorides), methanol (diethyltin chloride), or 1 M HCl (tin(IV) chloride). All other chemicals were reagent grade.

Extraction Efficiency

Fresh *S. alterniflora* leaves (from Sapelo Island, GA), roots, and rhizomes (both from Chapman's Landing, NH) were each cut into 1 cm segments, mixed, and ground to a fine powder under liquid nitrogen. Samples (1 g) of each matrix type were placed in polyallomer centrifuge tubes. To each sample between 0.85 and 1.0 ml H_2O and *ca.* 200 ng MeSn standards were added. Porewater samples (Chapman's Landing) were placed in scintillation vials with *ca.* 200 ng MeSn standards. After vortexing (VWR Vortexer II) the samples on high for 30 s they were left at room temperature overnight to allow tin compounds to incorporate into the plant matrix. The following day enough extractant was added to bring the sample volume to 5 ml. Porewater samples were acidified to 1 M HCl. All samples were sonicated for 1 h at 50°C then centrifuged (7500 rpm) for 15 min. The supernatant was decanted and analyzed via hydride generation atomic absorption spectrometry (HG-AAS).

The extraction solutions used were generally dilute acid, often mixed in varying ratios with methanol (Tables 3.1, 3.2, and 3.3). The amounts listed in these tables are the final concentrations, accounting for the water already added to the samples. After several methods had been tried, the most promising extractant was used in triplicate, with triplicate analysis to determine an accurate yield and a measure of the precision of the method.

Table 3.1. Recovery (%) of added $MeSn^{a}$ from *S. alterniflora* leaves using several different extractants.

Extractant	$MeSn^{3+}$	Me_2Sn^{2+}	Me_3Sn^{+}
1 M HCl	23.2	83.0	82.4
2 M HCl	65.5	88.3	82.7
3 M HCl	63.8	81.8	78.4
6 M HCl	80.6	95.1	88.7
0.5 M HCl/67% MeOH[b] (v/v)	35.2	80.7	81.4
0.5 M HCl/50% MeOH (v/v)	55.8	93.1	87.9
1 M HCl/50% MeOH (v/v)	59.7	79.3	83.5
2 M HCl/50% MeOH (v/v)	64.7	89.5	85.6
1 M HCl/75% MeOH (v/v)	69.8	84.4	87.0
1 M HCl/50% HAc[c] (v/v)	59.9	92.8	84.2
1 M HNO_3	14.5	72.5	85.0

[a]40 ng ml^{-1} each as Sn.
[b]Methanol.
[c]Acetic acid.

Table 3.2. Recovery (%) of added MeSn[a] from *S. alterniflora* root samples using different extraction solutions.

Extractant	$MeSn^{3+}$	Me_2Sn^{2+}	Me_3Sn^{+}
1 M HCl	63.5	72.9	82.2
2 M HCl	72.7	100	87.4
3 M HCl	51.8	84.5	70.7
6 M HCl	73.6	100	88.6
1 M HCl/75% MeOH[b] (v/v)	31.1	100	94.7
1 M HCl/50% HAc[c] (v/v)	78.9	81.5	96.4

[a]40 ng ml^{-1} each as Sn.
[b]Methanol.
[c]Acetic acid.

Table 3.3. Recovery (%) of added $MeSn^{a}$ from *S. alterniflora* rhizomes using various extractants.

Extractant	$MeSn^{3+}$	Me_2Sn^{2+}	Me_3Sn^{+}
1 M HCl	35.6	81.6	82.5
2 M HCl	58.3	83.9	85.9
3 M HCl	82.4	100	87.4
1 M HCl/50% MeOH[b] (v/v)	52.7	86.1	83.8
1 M HCl/75% MeOH (v/v)	73.2	86.5	82.4
2 M HCl/50% MeOH (v/v)	73.6	101	84.0

[a]40 ng ml^{-1} of each as Sn.
[b]Methanol.

Sample Collection

All samples were collected weekly at low tide for 13 weeks from Chapman's Landing on the Squamscott River which is part of the Great Bay estuarine system. Porewater was obtained from depths of 1-4 cm, 3-6 cm, 5-8 cm, and 7-10 cm below sediment surface with *in situ* "sippers" described by Hines *et al.* (1989). The samples were placed in glass scintillation vials and acidified to pH 1.0 with HCl upon arrival at the lab to prevent adsorption of the MeSn to the container walls.

A 225 cm^2 area (15 cm x 15 cm) was chosen from which to obtain the *S. alterniflora* leaves, roots, and rhizomes. Ten leaves from ten different plants were selected, cut into 1 cm segments, and mixed together. The roots and rhizomes were removed from the sediment, rinsed in water and separated from each other. Each sample was then homogenized by cutting into small (<0.5 cm) segments and shaking in a plastic bag. A portion, *ca.* 1-2 g, of each sample was immediately placed in an oven at 60°C for a week to determine dry weight/fresh weight ratios. The average dry weight/fresh weight ratios were 0.266 ± 0.054 for leaves, 0.239 ± 0.073 for rhizomes, and 0.188 ± 0.038 for roots. The remainder of the samples were stored in a freezer until analyzed.

Each week, five complete plants were cut at the air/sediment interface and the average plant mass and length were measured. Plant height increased from 14.2 cm at the beginning of the growing season to 157.6 cm. The average above-ground plant mass

increased from 0.39 g to 18.6 g during that same time. The plants reached their maximum height and mass by August12.

Sample Digestion

One gram samples were ground to a fine powder in liquid nitrogen using a mortar and pestle and placed in 30 ml polyallomer centrifuge tubes. To each sample 5 ml of the extraction solution shown to be the most effective for that particular matrix (Table 3.4) was added. All samples were then sonicated for 1 h at 50°C and centrifuged (7500 rpm) for 15 min. The resulting solution was decanted from the remaining solid and stored in a freezer (-10°C) until analyzed. Porewater samples (4.6 ml) were placed in scintillation vials, acidified to 1 M HCl through the addition of 0.4 ml concentrated HCl and stored at -10°C until analyzed.

Determination of Methyltin and Inorganic tin Compounds

Concentrations of tin compounds were determined as tin atoms by hydride formation, cryogenic trapping, boiling point separation and atomic absorption spectrophotometric detection. A more complete description of the instrumental method appears in Chapter I.

Table 3.4. Extraction media for *Spartina alterniflora* and porewater.

Sample (amount)	Extractant volumes	Final Molarity of Extractants	Analyte	% Recovery[a] ± 1 standard deviation[b]
Leaf (1 g)	0.85 ml water;	1 M HCl; 75 %	$MeSn^{3+}$	70 ± 1
	3.75 ml methanol;	methanol (v/v)	Me_2Sn^{2+}	84 ± 3
	0.40 ml 12 M HCl		Me_3Sn^{+}	87 ± 4
Rhizome (1g)	1.0 ml water;	3.0 M HCl	$MeSn^{3+}$	83 ± 3
	4.0 ml 3.75 M HCl		Me_2Sn^{2+}	135 ± 1
			Me_3Sn^{+}	87 ± 4
Root (1 g)	1.0 ml water; 1.5 ml	1.0 M HCl;	$MeSn^{3+}$	79 ± 1
	3.33 M HCl;2.5 ml	8.7 M acetic acid	Me_2Sn^{2+}	82[c]
	17.4 M acetic acid		Me_3Sn^{+}	96[c]
Porewater (4.6 ml)	0.4 ml 12.1 M HCl	1 M HCl	$MeSn^{3+}$	74 ±2
			Me_2Sn^{2+}	101 ± 2
			Me_3Sn^{+}	99 ± 4

[a] Based on 40 ng ml^{-1} as Sn.

[b]Standard deviation for extraction procedure. Separated from measurement error by an analysis of the variance components in an ANOVA table.

[c] Variance in extraction cannot be seen due to the large variance in measurement.

Results

Extraction Efficiency

Recoveries using the various extraction soutions are presented in Tables 3.1 (leaves), 3.2 (roots), and 3.3 (rhizomes). Table 3.4 shows the recoveries from each matrix along with the extraction variability as determined from an ANOVA of the nested design using the extraction solution deemed to be most effective.

Methyltin

All $MeSn^{3+}$ concentrations for leaves, roots, and rhizomes in Table 3.5 represent the average of two subsamples and three determinations of each. Two-way analysis of variance (ANOVA) calculations of the $MeSn^{3+}$ concentrations in porewater with respect to date and depth showed no difference at the 95% confidence level with depth ($F = 1.45$, degrees of freedom (df) = 3, probability $(P) > F = 0.2350$). We pooled values resulting from four depths (except for May 20), including at least two AAS determinations of each. The average relative standard deviations (RSD) reported resulted from within sample and between sample determinations. Within sample deviation represents the average error in the two or three AAS determinations of identical subsamples. Between sample deviation arises from the difference

Table 3.5. Concentrations of monomethyltin in *Spartina alterniflora* leaves, roots, and rhizomes (ng g^{-1} fresh weight) and mono- and dimethyltin in underlying porewater (ng ml^{-1}).

Date	$MeSn^{3+}$ Leaves[a]	$MeSn^{3+}$ Roots[a]	$MeSn^{3+}$ Rhizomes[a]	$MeSn^{3+}$ Porewater[b]	Me_2Sn^{2+} Porewater[b]
07 May	30.6	15.2	43.4	0.74	2.08
14 May	12.0	nd[c]	5.75	0.46	0.27
20 May	4.97	9.91	4.16	0.22	0.23
27 May	2.82	80.6	nd	0.40	0.29
03 June	5.48	nd	3.77	0.43	0.24
10 June	1.38	18.8	nd	0.39	0.27
17 June	2.85	30.2	2.97	2.61	0.23
24 June	8.93	28.7	15.1	0.98	0.23
01 July	1.83	26.5	3.06	0.56	0.20
08 July	10.2	nd	2.75	0.50	nd
15 July	16.6	19.6	3.05	0.27	nd
22 July	1.17	nd	3.62	nd	nd
29 July	3.77	nd	3.41	0.62	nd
Median	4.97	15.2	3.41	0.46	0.24
Average RSD:					
within sample	15.2	31.1	16.8	22.2	25.2
between samples	46.6	42.4	25.8	49.9	53.5
LOD[d] (ng g^{-1})	1	5	1	0.2	0.2

[a] Values represent 2 samples, 3 runs on each sample.
[b] Values represent 4 samples, 2 runs on each sample.
[c] nd = not detected.
[d] LOD = 3 times standard deviation of blank analysis.

in the two solid subsamples or the four porewater depths. The average deviation from all samples of a particular type is listed.

$MeSn^{3+}$ was found in all of the plant matrices and in the porewater nearly every week (Table 3.5). Me_2Sn^{2+} was found only occasionally in the solid samples (not in Table 3.5), but regularly in the porewater. In leaves $MeSn^{3+}$ concentrations ranged from 30.6 ng g^{-1} on May 7 to 1.17 ng g^{-1} on July 22, with a median of 4.97 ng g^{-1}. $MeSn^{3+}$ concentrations in roots range from undetected (5 ng g^{-1}) to 80.6 ng g^{-1} and had a median concentration of 15.2 ng g^{-1}. The median $MeSn^{3+}$ concentration in rhizomes was 3.41 ng g^{-1} with a range of 43.4 ng g^{-1} on May 7 to undetected (1 ng g^{-1}). $MeSn^{3+}$ concentrations in the porewater ranged from undetected (0.2 ng g^{-1}) to 2.61 ng g^{-1}, with a median concentration of 0.46 ng g^{-1}. The median Me_2Sn^{2+} concentration in porewater was 0.23 ng g^{-1} and the range was from undetected (0.2 ng g^{-1}) to 2.08 ng g^{-1}. The apparent order of $MeSn^{3+}$ concentrations in *S. alterniflora* and surrounding porewater was: roots > leaves ≈ rhizomes > porewater.

In order to determine the significance of the apparent difference among $MeSn^{3+}$ concentrations in the various plant parts from week to week, the Kruskal-Wallis Test (Miller and Miller, 1988) (Appendix A) was used. The Kruskal-Wallis Test is a ranking test for non-parametric data. Since the $MeSn^{3+}$ concentrations determined for each plant part on a weekly basis were not normally distributed, nor were the standard deviations of the weekly distributions statistically identical, we used the Kruskal-Wallis Test to rank the $MeSn^{3+}$ concentration among plant parts on each date to indicate whether there was a consistant trend

in the ranking. The resulting probability (P) is the probability that the null hypothesis is true (i.e. that the two samples are statistically equivalent). Tests of weekly concentrations with respect to plant part indicated a significant difference in the concentrations (chi-squared (χ^2) = 28.982, degrees of freedom (df) = 3, P = 0.0001).

The Wilcoxon Signed-Ranks method (STATA, 1990) (Appendix A) is a test for equality of distribution between two samples of non-parametric data. Wilcoxon Signed-Ranks analysis of the data showed that the highest MeSn concentrations for the majority of the days sampled were in the root samples. It was significantly greater than the concentrations detected in the leaf samples (z-statistic (z) = 2.10, P = 0.0357). The $MeSn^{3+}$ concentrations in the rhizomes is approximately equal to that in the leaves. (z = 0.44, P = 0.6566). The concentration of $MeSn^{3+}$ in the porewater is statistically less than the $MeSn^{3+}$ concentrations found in the rhizomes (z = 2.80, P = 0.0051). In the porewater, the concentration of Me_2Sn^{2+} were significantly less than the $MeSn^{3+}$ concentration (z = 3.58, P = 0.0003).

Average $MeSn^{3+}$ concentrations (Figure 3.1 and Table 3.5) showed neither a clear temporal trend for any of the sample types, nor any apparent relationship between the trends of the different sample types. ANOVA calculations indicated that there was a real difference beyond experimental error in the $MeSn^{3+}$ concentrations with respect to time for the *S. alterniflora* leaves and rhizomes (Table 3.6). By using regression analysis of the ANOVA output we can determine dates having concentrations statistically different from the median

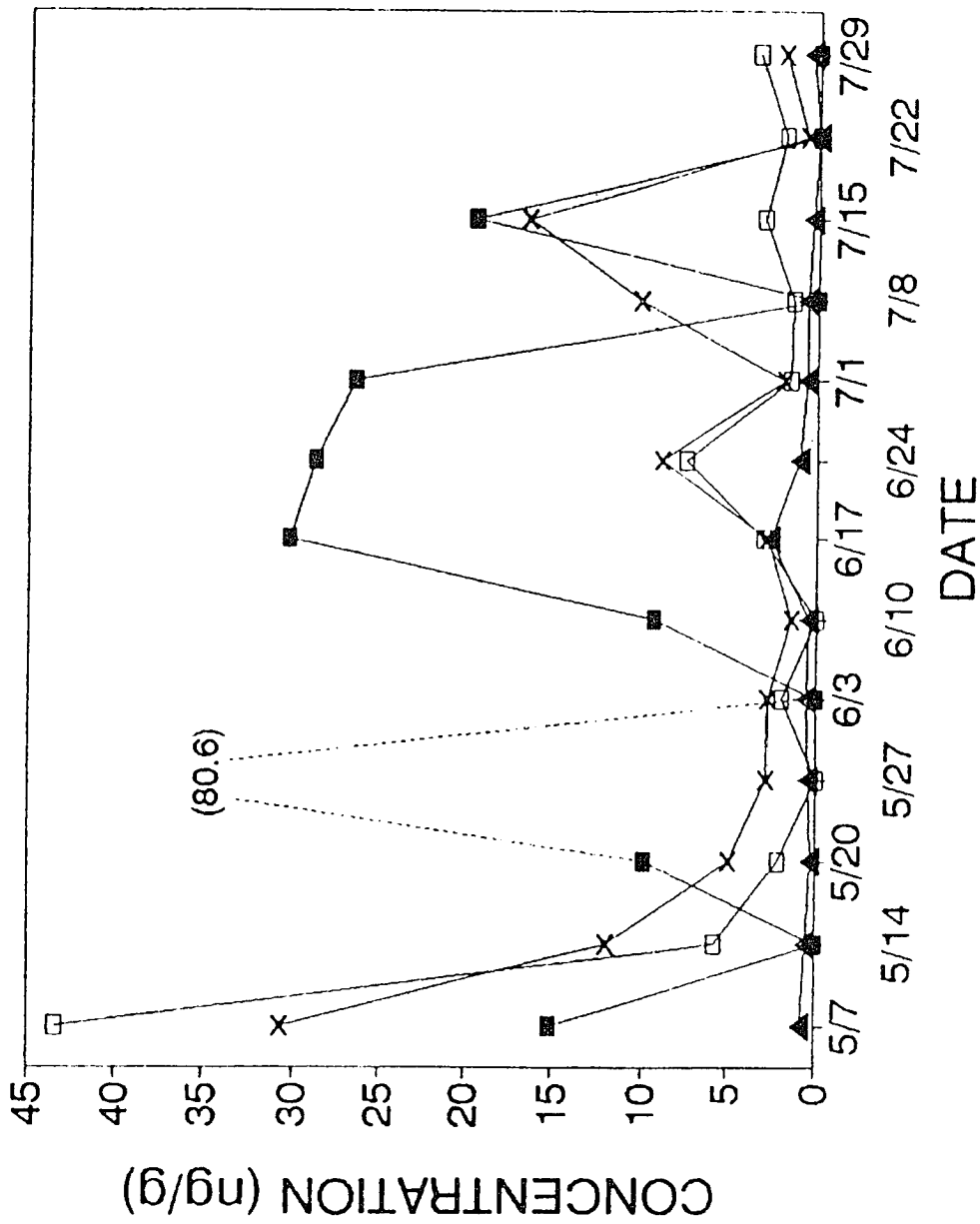

Figure 3.1. Averaged monomethyltin concentrations in leaves (X), roots (■), and rhizomes (□) of *S. alterniflora,* and surrounding porewater(▲) by date.

Table 3.6. Results of ANOVA[a] calculations of inorganic tin and monomethyltin concentrations within the various plant parts and porewater[b] with respect to date.

Sample Matrix	Sn	$MeSn^{3+}$
Leaf:		
F	30.72	46.09
df[c]	12	12
P > F[d]	0.0000	0.0000
Root:		
F	3.62	1.47
df	12	7
P > F	0.0004	0.2479
Rhizome:		
F	5.02	43.85
df	12	12
P > F	0.0000	0.0000
Porewater:		
F	8.52	0.94
df	12	11
P > F	0.0000	0.5110

[a] STATA program, Computing Resource Center (1990).
[b] Underlined values are significant at the 95% confidence level.
[c]Degrees of freedom.
[d]Probality that the null hypothesis is true.

value. Two significant peaks appearred in the leaf [$MeSn^{3+}$], one at the beginning of the growing season on May 7 (30.6 ng g^{-1}, P = 0.000) and the other on July 15 (16.6 ng g^{-1}, P = 0.010). The $MeSn^{3+}$ concentrations in the rhizomes on May 7 (43.4 ng g^{-1}, P = 0.000) and on June 24 (15.1 ng g^{-1}, P = 0.040) were significantly greater than at any other time during the growing season.

Inorganic Tin

Inorganic tin was found in all sample matrices on all dates sampled during the 1991 growing season (Table 3.7). It is present in much greater concentrations than $MeSn^{3+}$ (Table 3.5). Inorganic tin concentrations in *S. alterniflora* leaves ranged from a high of 502 ng g^{-1} on the first sampling date (May 7) to a low of 27.5 ng g^{-1} only a few weeks later on May 20, with a median concentration for the period observed of 53.8 ng g^{-1}. In roots inorganic tin concentrations ranged from a low of 335 ng g^{-1} in the beginning of the sampling season on May 14 to a high of 1320 ng g^{-1} on the final date of sampling, July 29, with a median concentration of 473 ng g^{-1}. *S. alterniflora* rhizomes contained between 107 ng g^{-1} inorganic tin on June 24 and 542 ng g^{-1} a few weeks earlier on June 10 and had a median inorganic tin concentration of 201 ng g^{-1}. ANOVA calculations of inorganic tin concentrations in porewater with respect to date and depth showed no difference in depth at the 95% confidence level (F = 2.17, P = 0.0968). The median inorganic tin concentration found in the

Table 3.7. Concentrations of inorganic tin (Sn) in *Spartina alterniflora* leaves, roots, and rhizomes (ng g^{-1}) and underlying porewater (ng ml^{-1}).

Date	Leaves[a]	Roots[a]	Rhizomes[a]	Porewater[b]
07 May	502	671	199	13.2
14 May	29.2	335	201	2.36
20 May	27.2	475	260	1.57
27 May	52.0	458	135	2.08
03 June	53.8	1110	239	1.86
10 June	58.0	457	542	2.00
17 June	84.6	422	178	21.3
24 June	58.4	473	107	9.61
01 July	108	455	318	8.11
08 July	29.7	1170	123	8.68
15 July	31.2	381	315	7.81
22 July	45.4	576	204	9.10
29 July	58.4	1320	132	8.73
Median	53.8	473	201	8.11
Average RSD:				
within sample	18.8	19.0	14.4	13.6
between samples	31.5	45.3	45.5	32.4
LOD (ng g^{-1})	1	1	1	0.2

[a] Values represent 2 samples, 3 runs on each sample.
[b] Values represent 4 samples, 2 runs on each sample.

porewater was 8.11 ng ml^{-1} and the concentrations ranged between 1.57 ng ml^{-1} on May 20 and 21.3 ng ml^{-1} on June 17.

We used the Kruskal-Wallis Test to ascertain any real differences in inorganic tin concentrations in each plant part and porewater when all four matrices were compared. Tests of the weekly concentrations with respect to sample matrix revealed a significant difference in the concentrations (χ^2 = 43.883, df = 3, P = 0.0001). To determine which of the sample matrices were different from the others we used the Wilcoxon Signed-Ranks Test. Similar to the trends in $MeSn^{3+}$ concentrations, the roots seemed to have the greatest inorganic tin concentrations. These were significantly greater than inorganic tin concentrations in rhizomes (z = 3.04, P = 0.0024). *S. alterniflora* leaves had significantly lower concentrations of inorganic tin than rhizomes (z = 2.34, P = 0.0192). The inorganic tin concentrations in the porewater were significantly lower than those in the leaves (z = 3.18, P = 0.0015). The order of inorganic tin concentrations in *S. alterniflora* and surrounding porewater was: roots > rhizomes > leaves > porewater.

There was no apparent temporal trend in average inorganic tin concentrations within any of the sample types, nor was any temporal relationship between the trends of different sample types discernable (Figure 3.2). ANOVA calculations of inorganic tin concentration with respect to date (Table 3.5) indicated that for each sample type there was a significant difference in concentrations by date. We again used a regression analysis of the ANOVA output to ascertain statistically different concentrations. The only

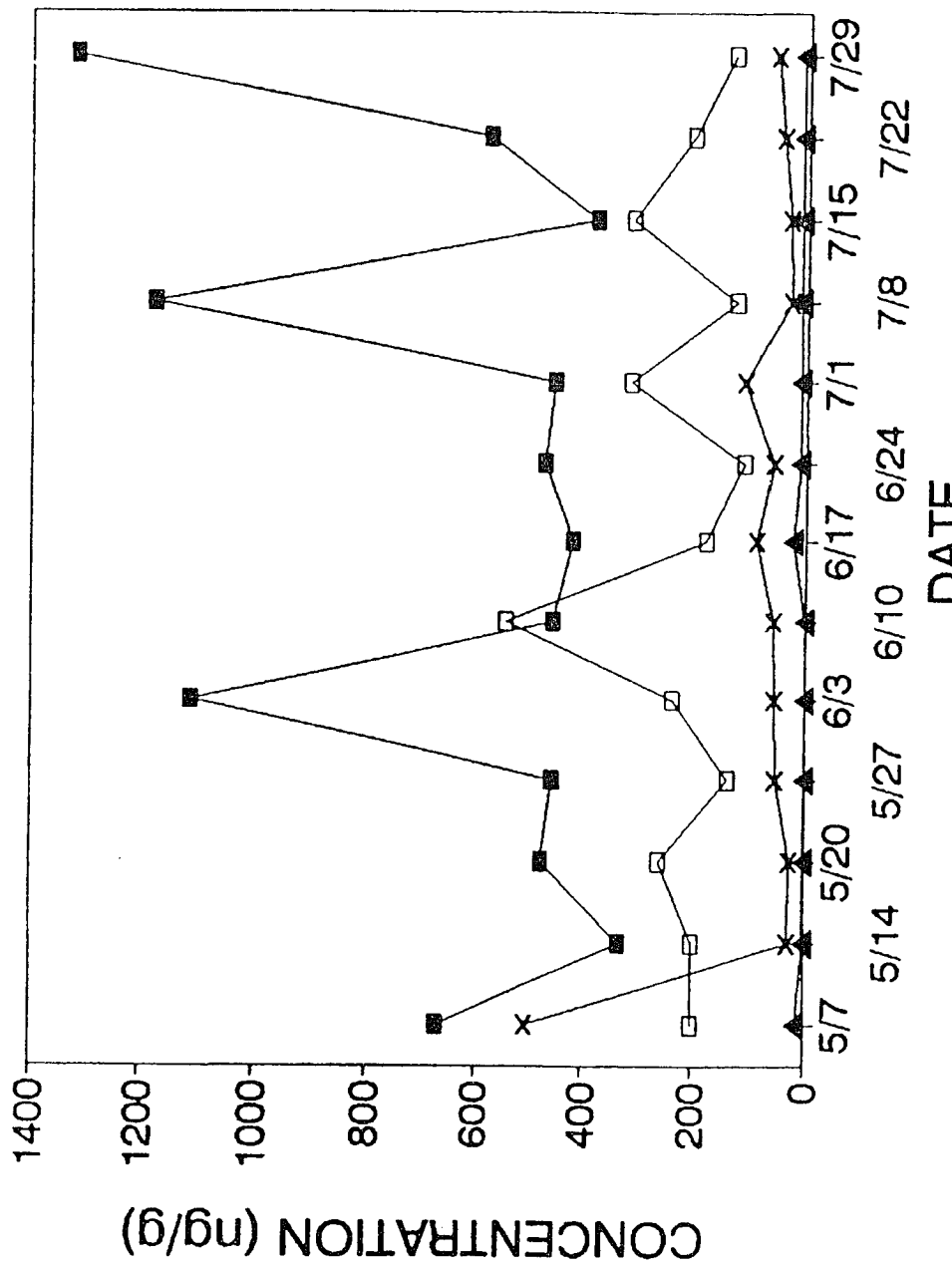

Figure 3.2. Averaged inorganic tin concentrations in leaves (X), roots (■), and rhizomes (□) of *S. alterniflora*, and surrounding porewater(▲) by date.

statistically different concentration in leaves occurred on May 7 (502 ng g^{-1}, P = 0.000). The roots displayed peaks of greater concentration on June 3 (1110 ng g^{-1}), July 8 (1170 ng g^{-1}, P = 0.002) and July 29 (1320 ng g^{-1}, P = 0.002). These could be the result of deviations that were not measured (*i.e.* tidal differences or increased rainfall). The significant peaks in the rhizome inorganic tin concentrations occurred on June 10 (542 ng g^{-1}, t = 5.274, P = 0.000), July 1 (318 ng g^{-1}, P = 0.012), and July 15 (315 ng g^{-1}, P = 0.014). The inorganic tin concentrations in porewater from May 14 through June 10 (avg. 1.97 ± 0.29) were statistically less than those from June 24 through July 29 (mean = 8.67 ± 0.65). On May 7 the concentration (13.2 ng g^{-1}) was significantly different (t = 3.184, P = 0.002) from the median concentration (8.11 ng g^{-1}).

Discussion

Extraction Efficiency

The first problem was to find methods to extract, quantitatively, methyltin compounds from leaves, root, and rhizomes of *S. alterniflora* and surrounding porewater. Many researchers extract environmental samples by hot acid digestion, but this method could possibly break carbon-tin bonds and prevent speciation of methyltin compounds and inorganic tin. Even though extraction with 6 M HCl gave the greatest yields of MeSn, other problems and potential problems prevented its use. During the hydride generation phase of

the analysis, addition of $NaBH_4$ caused excessive effervescence necessitating the use of a surfactant. Also, 6 M HCl is a very strong acid that could possibly, over time, result in scission of the tin-carbon bond or cause disproportionation reactions to occur. Each matrix (Table 3.4) required a different extractant for optimum percent recovery of a 40 ng g^{-1} spike. $MeSn^{3+}$ is always the most difficult of methyltin compounds to extract, but the average recovery was 70% and errors were reasonable. Therefore, 1 M HCl/75% MeOH (v/v) is the extractant of choice for *S. Alterniflora* leaves. The best extraction of those tried of MeSn from roots was possible using a solution of 1 M HCl/50% acetic acid (HAc). Quantitative extraction of MeSn from rhizomes was best achieved using 3 M HCl.

Absence of Temporal Trends in Concentrations of Methyltin Compounds and Inorganic Tin

$MeSn^{3+}$ concentrations (Figure 3.1) in *S. alterniflora* leaf tissue did not show a strong temporal trend in contrast to our previous study (Weber *et al.*, 1991); concentrations in rhizomes, roots, and porewater also followed a similar pattern from week to week. This contradicts our previous hypothesis (Weber *et al.*, 1991) that high $MeSn^{3+}$ concentrations in leaves early in the growing season were linked to uptake of stored carbohydrates from rhizomes. If $MeSn^{3+}$ followed this pattern, its concentration would increase in leaf tissue while decreasing in rhizomes. However, $MeSn^{3+}$ concentrations in leaves and rhizomes followed parallel trends.

Concentrations of Methyltin Compounds and Inorganic Tin in Parts of *S. alterniflora* and Surrounding Porewater

MeSn and inorganic tin could enter plants either via estuarine water or sediments. We believe that absorption from water is the less likely mode because concentrations of MeSn were much higher in sediments than in water (Thompson *et al.*, 1985; Maguire *et al.*, 1986; Maguire, 1991) and because the plants were under water only about two hours per day at high tides. Therefore, the following discussion assumes absorption by roots.

There are two possible origins of MeSn in roots. Either MeSn in the porewater/sediment is absorbed by roots and/or inorganic tin is absorbed into the roots and methylated there. It seems reasonable that MeSn originate directly from porewater/sediments where their concentration is relatively high. More convincingly, there were no patterns in the roots' data that support increased concentrations of inorganic tin (Figure 3.2) coincident with or followed by increased MeSn concentrations (Figure 3.1). These results suggest that MeSn in the plants originate from their uptake by roots rather than from methylation of inorganic tin within the plant.

Observations that $MeSn^{3+}$ (Figure 3.1 and Table 3.5) and inorganic tin (Figure 3.2 and Table 3.7) concentrations were greatest in the roots at almost all times during the growing season demonstrate poor translocation of MeSn from roots to rhizomes and leaves. Higher concentrations of inorganic tin in roots than leaves agree with Weber and Alberts'

(1990) study of uptake of inorganic tin by hydroponically grown *S. alterniflora*. When the inorganic tin concentration in the nutrient solution was 50 ng ml^{-1}, the concentration factor for inorganic tin (concentration in plant part/concentration in solution) averaged 18 for roots and 3.2 for leaves. Thus, inorganic tin is not effectively translocated from solution through roots to leaves. Alberts *et al.* (1990) observed poor translocation from roots to other tissues of *S. alterniflora* for three (Al, Fe, and Zn) of six metals taken up by the plants from sediments.

Low concentrations of $MeSn^{3+}$ in porewater (Figure 3.1 and Table 3.5) may reflect its low solubility in water or strong adsorption by sediments. The fact that the median concentration of Me_2Sn^{2+} in porewater is about 50% that of $MeSn^{3+}$ (Table 3.5) is not surprising because its formation requires a second methylation step. The conversion of $MeSn^{3+}$ to Me_2Sn^{2+} by the usual method of oxidative addition is not possible without the unlikely previous reduction of $MeSn^{3+}$ to a methylated Sn(II) compound.

Clearly this study of $MeSn^{3+}$ and inorganic tin in parts of *S. alterniflora* and surrounding porewater, which is the only study of its kind, is insufficient to unravel the biogeochemical complexities of the tin cycle in *S. alterniflora* salt marshes. Future studies should focus on the rhizosphere of *S. alterniflora*.

CHAPTER IV

METHYLATION OF INORGANIC TIN BY DECAYING *Spartina alterniflora* IN ESTUARINE WATER, AND BY ESTUARINE WATER

Introduction

Donard *et al.* (1987) studied the effect of the green algae, *Enteromorpha,* on the formation and stability of methyltin compounds (MeSn). They found that mono-, di-, and trimethyltin compounds were produced as the algae decomposed. They also found that several seaweeds are capable of concentrating MeSn relative to the surrounding estuarine water. Wright and Weber (1991) investigated the rates of accumulation of inorganic tin and MeSn by *Fucus vesiculosus* and a mixed community of *Enteromorpha* as these macroalgae decomposed. They found that all four MeSn and Sn were biosorbed by both species of algae. The rate of accumulation consisted of three distinct phases: (1) a rapid initial phase associated with surface adsorption; (2) a moderately fast second phase attributed to an extracellular process; and (3) a slow final phase of cellular accumulation. No study, thus far, has investigated the role of decaying *Spartina alterniflora* in the cycling of tin and MeSn.

Weber and Alberts (1990) previously demonstrated that hydroponically grown *S. alterniflora* accumulate monomethyltin (Me_3Sn^+) from Sn(IV) amended media, but several unpublished studies in this laboratory on the ability of decaying *S. alterniflora* to methylate

and demethylate tin have been inconclusive. For example, Proulx-Curry (personal communication) studied decaying *S. alterniflora* leaves, roots, and rhizomes in estuarine water that had been amended with low (5 ng ml^{-1}) and high (150 ng ml^{-1}) concentrations of Sn. In some of the plant parts incubated in tin amended estuarine water she found high MeSn concentrations, however, the plants spiked with larger amounts of Sn did not exhibit higher concentrations of MeSn. Possibly, high Sn concentrations significantly altered the chemistry of the samples and/or killed all but the tin resistant bacteria, thus eliminating some of the methylating bacteria. A possible conclusion is that methylation during the decay process resulted directly from a bacterial enzymatic process or from chemical(s) released or formed during the decay process.

No published study has examined the role of decaying *S. alterniflora* in the cycling of tin compounds. In this study, we examined the role of decaying *S. alterniflora* leaves in the uptake of inorganic tin and the subsequent formation of MeSn. Decaying *S. alterniflora* leaves concentrate Sn from surrounding waters. A significant decrease in dimethyltin (Me_2Sn^{2+}) accompanied by a significant increase in Me_3Sn^+ indicate MeSn rearrangement in conjunction with the decay process. Because of the importance of *S. alterniflora* in estuaries, formation of MeSn during their decay may form a significant contribution to MeSn found in the estuaries.

This chapter was previously published by Falke and Weber (1994).

Experimental

Materials

All fresh water used was doubly deionized and distilled through a Corning Mega-pure still. $MeSn^{3+}$, Me_2Sn^{2+}, trimethyltin (Me_3Sn^{+}), and inorganic tin(IV) chlorides (97+ % purity) were obtained from Alfa Chemicals. Stock solutions of *ca.* 1000 mg ml^{-1} (all concentrations as Sn) were prepared in 1 M HCl. Standards were made by diluting stock solutions to *ca.*. 5 mg ml^{-1} in 0.05 M HNO_3 (methyltin chlorides) or 1 M HCl ($SnCl_4$). Sodium borohydride ($NaBH_4$) solution (6%) was prepared by dissolving 12 g $NaBH_4$ (Aldrich Chemicals, 98%) in 100 ml water and allowing to stand, refridgerated, overnight. The solution was then filtered through a 0.2 mm polycarbonate filter (Nuclepore) to remove any Sn colloids, and diluted to 200 ml with water. All other chemicals were of reagent grade. All glassware and plasticware were soaked overnight in a 10% HNO_3 solution. The containers used for the experiments were 1000 ml Florence flasks modified with a nozzle at the base and connected to an air source. A porous frit separated the interior of the flask from the air source to prevent flow of flask contents into the air lines.

Sample Collection

Estuarine water was obtained in 2.5 L bottles off the dock at Jackson Estuarine Laboratory in the Great Bay Estuary (NH). To avoid collecting the top microlayer of water, which tends to have higher concentrations of inorganic tin and MeSn (Donard *et al.*, 1986), care was taken to uncover and cover the bottles at least 5 cm below the surface. The water was collected on the side of the dock from which the water was flowing to avoid any contamination from the dock. HG-AAS analysis of the water revealed an Sn concentration of 8.8 ng ml. No MeSn were detected. *S. alterniflora* leaves were collected in early July 1993 at Chapman's Landing in the Squamscott River (NH) by cutting leaves from the stalk. Leaves were cut into 0.5 cm lengths and mixed together to homogenize as much as possible.

Experiment 1: Decomposing *S. alterniflora* Leaves

Three flasks were each filled with 10 g homogenized leaf and 500 ml estuarine water. Each flask was spiked with 11.75 μg Sn (from $SnCl_4$ stock solution). House compressed air was first passed through a cotton plug to remove any oils or particulates and then bubbled through each flask at a rate just great enough to create a steady stream of small bubbles to keep the sample aerated. All flasks were placed on a laboratory bench exposed to indirect sunlight for *ca.* 16 h day^{-1}. Every 6 h for the first 30 h *ca.* 1 g leaf, weighed accurately, and 20.0 ml water were removed from each experiment. The water samples were

immediately acidified to pH 0 through addition of 1.8 ml 12.1 M HCl. Unamended estuarine water (20 ml) was added to each sample to replace that removed. Distilled water was added to replace any that had evaporated. Leaf samples were washed by shaking in a 10 ml aliquot of 0.05 M HNO_3 and the washing collected. Leaves were then rinsed with two 5 ml aliquots of water and dried by pressing lightly between two Kimwipes. Leaves were ground in liquid nitrogen and extracted in 1 M HCl; 75% (v/v) methanol (Falke and Weber, 1993).

Experiment 2: Methylation by Estuarine Water

Three flasks were filled with 500 ml estuarine water. To each flask 36.7 μg Sn (as above) and $MeSn^{3+}$, Me_2Sn^{2+}, and Me_3Sn^{+} (*ca.* 12.5 μg as Sn from stock solutions) were added. House air was bubbled through the experiments as in Experiment 1. Samples were again exposed to sunlight. Aliquots (20 ml) were removed immediately and again on days 2 and 4 from each flask. Each aliquot was immediately acidified to pH 0 and stored in the freezer until analyzed.

Determination of Inorganic tin and Methyltin Compounds

Determinations of Sn and MeSn compounds was performed by hydride generation followed by cryogenic trapping and chromatographic separation followed by atomic absorption spectrophotometry as described in Chapter II.

Results

Experiment 1: Decomposing *S. alterniflora* Leaves

Concentrations of inorganic tin in the estuarine water containing decomposing *S. alterniflora* leaves can be found in Table 4.1. Sn concentrations in estuarine water decreased from 28 ng ml^{-1} (mean at 6 h) to 13 ng ml^{-1} (mean at 120 h) during the 120 h studied. An analysis of variance (ANOVA) at the 95% confidence level indicated that this decrease was significant ($F = 16.97$, degrees of freedom (df) = 6, probability (P) > F = 0.0000). $MeSn^{3+}$ and Me_2Sn^{2+} (Table 4.1) occurred occasionally in water samples, but with no temporal trend.

The initial concentration of inorganic tin in the leaves (Table 4.2) was *ca.* 28 ng g^{-1} There was approximately an order of magnitude increase in the concentration of Sn in the

Table 4.1. Variation in concentrations of inorganic tin and methyltin compounds with time, in estuarine water containing decomposing *S. alterniflora* leaves and supplemented with inorganic tin (32.5 ng ml^{-1})[a,b,c].

Time (h)	Concentration (ng ml^{-1}) ± 1 standard deviation Sn	$MeSn^{3+}$	Me_2Sn^{2+}
0[d]	2.08 ± 0.26	0.16 ± 0.004	1.4 ± 0.003
0[d]	1.43 ± 0.13	0.08 ± 0.01	0.49 ± 0.03
6	23.9 ± 0.88	D/ND	D/ND
6	32.0 ± 1.6	ND	ND
6	27.2 ± 3.2	0.594 ± 0.019	0.835 ± 0.094
12	22.6 ± 0.2	ND	ND
12	27.1 ± 2.7	ND	ND
18	32.2 ± 2.6	ND	ND
18	32.9 ± 4.2	ND	ND
18	33.2 ± 6.4	ND	ND
24	22.1 ± 1.2	ND	ND
24	26.5 ± 0.6	ND	0.506 ± 0.038
24	28.7 ± 1.2	ND	D/ND
30	18.2 ± 2.8	ND	ND
30	16.7 ± 1.3	0.61 ± 0.14	0.74 ± 0.16
30	13.3 ± 0.9	ND	ND
48	20.6 ± 2.4	2.04 ± 0.33	1.01 ± 0.25
48	15.9 ± 0.6	ND	D/ND
48	14.3 ± 1.8	ND	ND
120	15.3 ± 0.6	ND	ND
120	14.3 ± 0.5	ND	ND
120	9.8 ± 1.1	D/ND	0.70 ± 0.22

[a] ND: compound was not detected. Limit of detection (base line + 3σ) at t = 0 (sample volume = 5.0 ml) was 0.044 ng ml^{-1} and is 0.44 ng ml^{-1} at all other times (sample volume = 0.5 ml).

[b] D/ND: value was above the level of decision (baseline + 1.5 σ) but below the limit of detection.

[c] At each time, 3 different samples were analyzed. Each number represents the average of at least 2 determinations.

[d] Represents the concentration prior to the addition of inorganic tin spike.

Table 4.2. Variation of inorganic tin and methyltin concentrations in decomposing *S. alterniflora* leaves and leaf washings in estuarine water supplemented with 1.18 μg Sn per g of leaf material[a,b,c].

	Concentration (ng g^{-1} ± 1 standard deviation)							
	Within leaf				Leaf washings[d]			
Time(h)	Sn	$MeSn^{3+}$	Me_2Sn^{2+}	Me_3Sn^{+}	Sn	$MeSn^{3+}$	Me_2Sn^{2+}	Me_{tot}[e]
0[f]	34.2 ± 2.8	2.32 ± 0.08	10.6 ± 0.2	ND	NA	NA	NA	23.5
0[f]	24.8 ± 3.2	1.72 ± 0.18	7.69 ± 0.93	ND	NA	NA	NA	17.1
0[f]	29.7 ± 3.8	ND	ND	ND	NA	NA	NA	ND
0[f]	24.7 ± 5.8	ND	9.18 ± 0.51	ND	NA	NA	NA	18.4
6	125 ± 16	ND	ND	ND	2.66 ± 0.005	1.49 ± 0.004	4.59 ± 0.54	10.7
6	69.7 ± 8.7	ND	5.09 ± 0.43	ND	7.68	2.42 ± 0.16	7.42 ± 0.17	27.4
6	119 ± 17	ND	8.06 ± 0.95	ND	8.7 ± 1.6	31.1 ± 2.7	50.2 ± 0.7	148
12	193 ± 21	D/ND	12.0 ± 2.9	D/ND	7.69 ± 0.48	D/ND	D/ND	27.2
12	106 ± 20	D/ND	D/ND	D/ND	2.50 ± 0.08	D/ND	1.42 ± 0.06	3.99
12	114 ± 13	ND	ND	ND	2.34 ± 0.09	ND	1.46 ± 0.06	2.92
18	90.2 ± 0.1	ND	ND	ND	10.6	1.31 ± 0.15	1.60 ± 0.13	4.51
18	174 ± 11	12.8 ± 1.1	ND	ND	1.87 ± 0.26	1.71 ± 0.26	7.02 ± 0.54	28.6
18	144 ± 3	5.56	ND	ND	3.36 ± 0.58	21.6 ± 8.5	5.4 ± 1.6	37.9
24	148 ± 10	D/ND	ND	6.27 ± 0.54	3.05	D/ND	5.48 ± 0.72	30.3
24	182 ± 7	ND	ND	ND	6.93	1.29 ± 0.14	8.83 ± 0.50	19.0
24	215 ± 16	ND	ND	ND	2.94 ± 0.01	30.7 ± 2.2	29.8 ± 1.7	90.3
30	147 ± 20	D/ND	ND	11.4 ± 2.6	5.44	D/ND	4.70 ± 0.16	44.5
30	188 ± 23	D/ND	D/ND	D/ND	1.74 ± 0.75	D/ND	6.09 ± 0.29	13.1
30	226 ± 37	ND	ND	19.9 ± 0.4	1.41 ± 0.004	2.79 ± 0.60	10.2 ± 0.1	82.9
48	167 ± 4	ND	ND	ND	1.68 ± 0.08	D/ND	4.57 ± 0.58	10.3
48	364 ± 1	ND	D/ND	D/ND	1.70 ± 0.22	1.65 ± 0.36	9.99 ± 0.36	21.6
48	229 ± 19	ND	ND	ND	2.05	D/ND	6.70 ± 0.15	14.6

Table 4.2. Continued.

	Concentration (ng g^{-1} ± 1 standard deviation)							
	Within leaf				Leaf washings[d]			
Time(h)	Sn	$MeSn^{3+}$	Me_2Sn^{2+}	Me_3Sn^{+}	Sn	$MeSn^{3+}$	Me_2Sn^{2+}	Me_{tot}[e]
120	317 ± 35	6.45 ± 0.16	ND	12.68	6.96 ± 0.26	ND	D/ND	46.1
120	293 ± 34	ND	D/ND	D/ND	7.42	D/ND	D/ND	2.66
120	542 ± 80	D/ND	ND	D/ND	4.65 ± 0.22	ND	D/ND	1.28

[a]ND: compound was not detected. Limit of detection (baseline = 3σ) in the leaf washings (1.0 ml sample) is 1.25 ng g^{-1} and in the leaf samples (0.25 ml samples) was 5.0 ng g^{-1}.

[b] D/ND: value was above the limit of decision (baseline + 1.5σ), but below the limit of detection.

[c]At each time, three different samples were analyzed. Each number represents the average of at least two determinations.

[d]Leaves were not washed prior to commencing the experiment.

[e]$[Me]_{tot} = [MeSn^{3+}] + 2[Me_2Sn^{2+}] + 3[Me_3Sn^{+}]$ and includes both leaves and washings.

[f]Represents leaf concentrations prior to the addition of inorganic tin spike.

decaying leaves during the 5 days studied. This represented a statistically significant increase ($F = 8.56$, $df = 7$, $P > F = 0.0002$).

Prior to adding inorganic tin (t = 0) the $MeSn^{3+}$ concentration in the leaf samples was *ca.* 2 ng g^{-1} and that of Me_2Sn^{2+} was *ca.* 9 ng g^{-1}. No Me_3Sn^+ was detected in the leaf samples [limit of detection (LOD) = 0.44 ng g^{-1}]. After addition of Sn, $MeSn^{3+}$ occurs occasionally with no apparent trend (Table 4.2 and Figure 4.1). There was no statistically significant difference between the initial $MeSn^{3+}$ concentrations and later ones. Me_2Sn^{2+} was present at detectable concentrations quite regularly during the first 12 h of sampling and then concentrations drop below the limit of detection. Me_3Sn^+, on the other hand, was rarely seen during the first 18 h and was regularly detected after that. While both Me_2Sn^{2+} and Me_3Sn^+ exhibited apparent trends in concentration with time, the amount of actual data is insufficient to statistically support this observation. Based on assumptions that MeSn were present in most of the samples at concentrations below the limit of detection (LOD = 5 ng g^{-1}) and that analysis errors would be similar to that in the actual data, we fabricated "dummy" values for the undetected concentrations. That is, we replaced undetected concentrations of Me_2Sn^{2+} and $MeSn^{3+}$ with 3 values with concentrations below the LOD that had a 20% relative deviation. ANOVA of the data showed that both Me_2Sn^{2+} and Me_3Sn^+ exhibited a statistically significant trend with time (Me_2Sn^{2+}: $F = 6.97$, $df = 7$, $P>F = 0.0025$; Me_2Sn^{2+}: $F = 7.05$, $df = 7$, $P>F = 0.0236$).

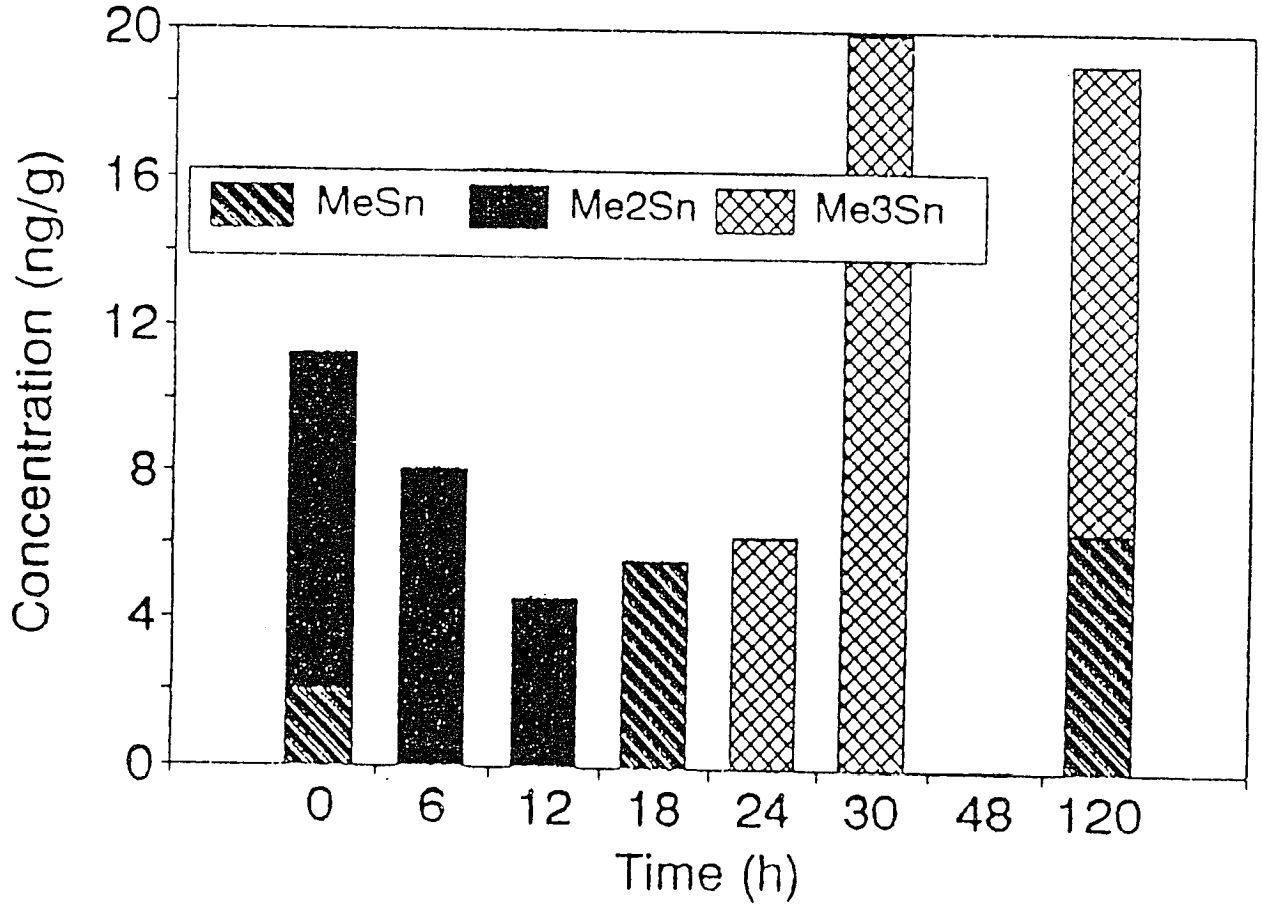

Figure 4.1. Variation of averaged methyltin concentrations in *S. alterniflora* leaves decaying in estuarine water with time after spiking with 1.17 μg g^{-1} inorganic tin.

Inorganic tin, $MeSn^{3+}$ and Me_2Sn^{2+} appearred regularly in the leaf washings (Table 4.2) with no apparent temporal trend. The mass of inorganic tin found in the wash accounted for only 2.2% of the total inorganic tin associated with the leaves, but the percent of $MeSn^{3+}$ (14%) and Me_2Sn^{2+} (38%) was more significant. The washings contained a significantly greater Me_2Sn^{2+} concentration than $MeSn^{3+}$ concentration (t-statistic = 4.14, df = 33, P>t = 0.0005).

Since there are 2 methyl groups for every Me_2Sn^{2+} and 3 methyl groups for every Me_3Sn^{+}, the total concentration of methyl groups bound to tin atoms ($[Me]_{tot}$ in leaves and washings is shown in Equation 4.1.

$$[Me]_{tot} = [MeSn^{3+}] + 2[Me_2Sn^{2+}] + 3[Me_3Sn^{+}] \qquad \text{(Equation 4.1)}$$

$[Me]_{tot}$ associated with the leaves did not vary with time with time (F = 1.11, df = 6, P>F = 0.4063).

Experiment 2: Methylation by Estuarine Water

Table 4.3 and Figure 4.2 show the concentrations of tin compounds in estuarine water samples (no leaves) that were spiked with inorganic tin (*ca.*75 ng ml^{-1}) and MeSn (*ca.* 25 ng ml^{-1}). The Sn concentration decreased substantially (F = 178.72, df = 2, P>F = 0.0000) during the 4 days of study. $MeSn^{3+}$ concentration (Figure 2) decreased on Day 2 to *ca.* 16 ng

Table 4.3. Variation in concentrations of inorganic tin (Sn) and methyltin compounds (MeSn) with time in estuarine water amended with Sn (75 ng ml^{-1}) and MeSn (25 ng each ml^{-1}).

	Concentration (ng ml^{-1}) ± 1 standard deviation				
Time (d)	Sn	$MeSn^{3+}$	Me_2Sn^{2+}	Me_3Sn^{+}	$[Me]_{tot}$ [a] (ng ml^{-1})
0	86.4 ± 0.6	23.1 ± 0.8	25.1 ± 0.7	27.3 ± 3.6	155
0	73.5 ± 0.7	22.6 ± 0.2	22.6 ± 1.5	26.6 ± 0.2	148
0	69.2 ± 0.5	18.6 ± 1.6	23.9 ± 0.9	29.3 ± 0.8	154
2	49.4 ± 0.7	17.0 ± 0.6	25.9 ± 2.6	28.8 ± 2.5	155
2	26.1 ± 0.5	15.5 ± 0.4	28.1 ± 0.3	30.8 ± 1.1	164
2	33.1 ± 5.9	16.5 ± 2.6	28.7 ± 0.8	31.4 ± 1.2	168
4	39.3 ± 0.1	22.1 ± 0.2	28.8 ± 1.1	32.0 ± 0.7	175
4	31.0 ± 2.9	19.5 ± 1.0	30.7 ± 0.2	33.3 ± 1.2	181
4	34.0 ± 3.4	15.8 ± 1.2	28.6 ± 2.6	30.7 ± 3.6	165

[a] Represents the sum of $[MeSn^{3+}] + 2[Me_2Sn^{2+}] + 3[Me^3Sn^{+}]$.

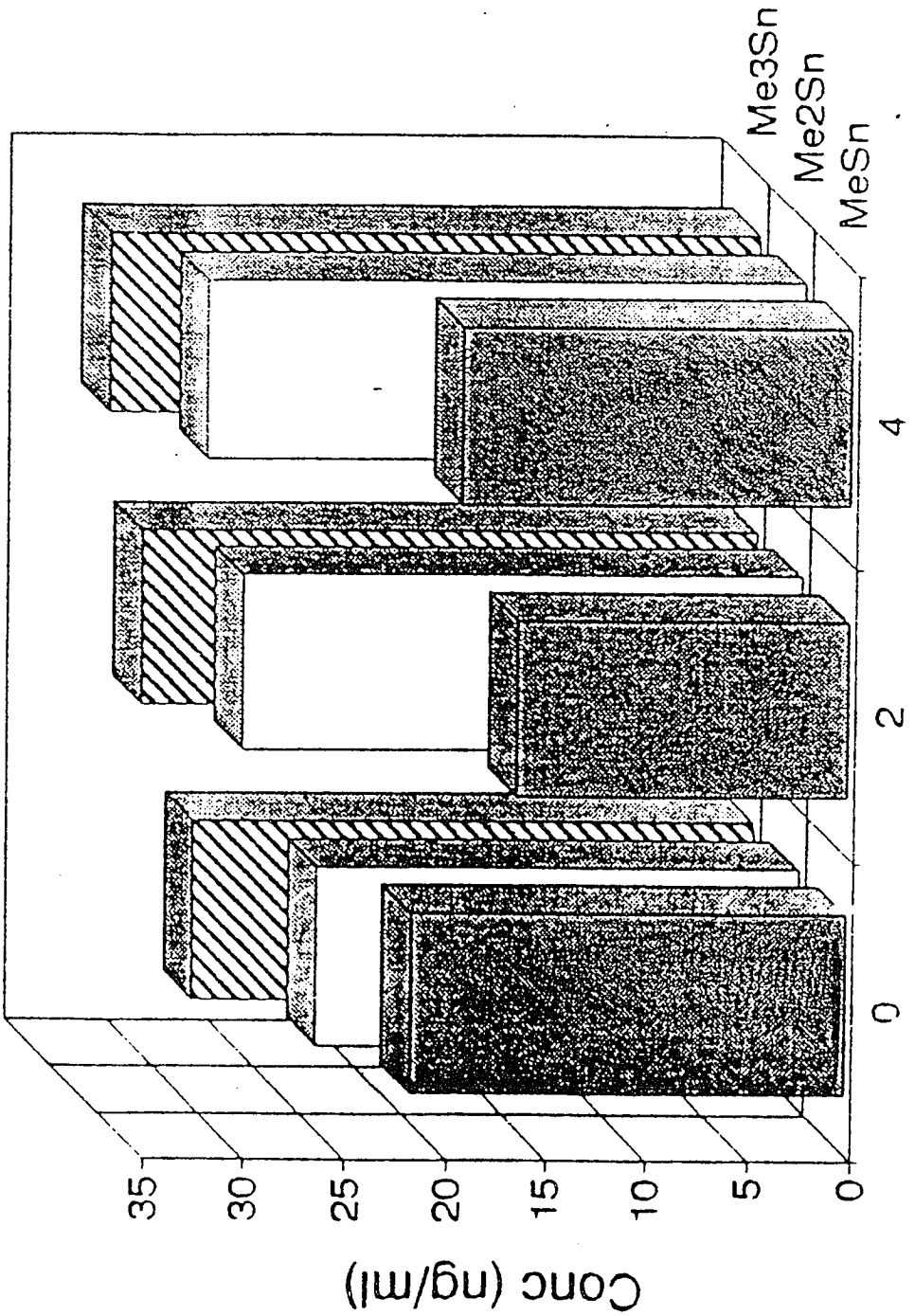

Figure 4.2. Variation of averaged methyltin concentrations in estuarine water with time after spiking with 75 ng ml^{-1} inorganic tin and 25 ng ml^{-1} $MeSn^{3+}$, Me_2Sn^{2+}, and Me_3Sn^{+}.

ml^{-1}, then returned to *ca.* 19 ng ml^{-1}. $MeSn^{3+}$ concentrations on Days 0 and 4 were significantly different from those of Day 2 ($F = 7.36$, $df = 2$, $P>F = 0.0050$). Me_2Sn^{2+} concentration increased significantly ($F = 17.24$, $df = 2$, $P>F = 0.0001$) from 24 ng ml^{-1} (Day 0) to 29 ng ml^{-1} (Day 4). The Me_3Sn^+ concentrations, which followed the same pattern as the Me_2Sn^{2+}, increased significantly ($F = 6.13$, $df = 2$, $P>F = 0.0099$) during the 4 day experiment. $[Me]_{tot}$ increased significantly with time ($F = 8.27$, $df = 2$, $P>F = 0.0189$).

Discussion

Decomposing *S. alterniflora* Leaves

In a preliminary study, we examined the effect of light and air on the methylation of Sn by decaying *S. alterniflora* leaves. Inorganic tin spikes ranged from 0 to 100 ng ml^{-1}. Plant samples maintained in a light/dark cycle and in an oxic environment seemed to produce higher concentrations of MeSn, however the difference was not statistically significant. As a result, we eliminated light and air as possible factors and ran all experiments in an oxic environment under a natural light/dark cycle. An additional experiment confirmed that bubbling air through the samples caused no loss of MeSn due to volatilization. Thus, any loss of MeSn observed resulted from methylation and/or demethylation processes.

The concentration of the inorganic tin spike in this experiment was chosen as 23.5 ng ml^{-1} for two reasons. Typical tin concentrations in the Great Bay Estuary are in the low ng ml^{-1} range (Donard *et al.*, 1986). The estuarine water sample collected had an inorganic tin concentration of 2.39 ng ml^{-1}. The detectable concentration in environmental samples typically had about 20 % error associated with it. We wanted to be able to detect changes in the spiked concentration at better than 90% accuracy, but still be within realistic environmental concentrations. A higher concentration would allow for more accurate detection, however, that would then place the spiked concentration higher than that normally found in estuarine water samples.

It was not surprising that the inorganic tin concentration in the estuarine water above decomposing *S. alterniflora* leaves decreased with time. The pH of estuarine water is *ca.* 7.0 and inorganic tin is known to adsorb strongly to container walls at that pH. At the end of the decomposing leave experiment, roughly 30% of the added Sn is associated with the plant leaves (Table 4.3), and *ca.* 50% remains in solution (Table 4.2). This left about 20% of added tin that adsorbed to walls of the flask or became otherwise undetectable.

The increase in inorganic tin concentration in the leaves was also predictable. Huang *et al.*(1993) found that *S. alterniflora* concentrates metals from the surrounding water column. Additionally, Alberts *et al.* (1990) found that the elemental concentration of many metals increases in decaying *S. alterniflora* leaves. Algae also tend to bioconcentrate Sn as it decays from the surrounding water column. Wright and Weber (1991) determined the uptake

of inorganic tin onto two different marine algae, *Fucus vesiculosus* and a mixed community of *Enteromorpha*. They found a three-phase biosorption process. The first phase was extremely fast and they were unable to determine rate constants. Phase 2 was slower with pseudo-first-order rate constants of 0.86 h^{-1} for *F. vesiculosus* and 0.65 h^{-1} for *Enteromorpha*. Phase 3 was associated with cellular accumulation. It was the slowest phase in the biosorption process with a rate of 350 ng g^{-1} h^{-1} for *F. vesiculosus* and 4 ng g^{-1} h^{-1} (not significantly greater than zero, P = 0.05) for *Enteromorpha*.

Biosorption of Sn by *S. alterniflora* (Table 4.2) did not continue until all Sn had been adsorbed, but by 120 h the rate of adsorption began to stabilize. Because inorganic tin remains in the water, the process must be in equilibrium (Equation 4.3).

$$[Sn]_{water} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Sn]_{leaves} \qquad \text{(Equation 4.2)}$$

It can be treated as opposing pseudo first order reactions. The integrated rate equation is analgous to that used for unidirectional reactions, but the derived first order rate constant (k) is the sum of the foward and reverse rate constants ($k_1 + k_{-1}$) (Wilkins, 1974). The integrated rate equation for first-order or pseudo-first-order kinetics is:

$$\ln ([Sn]_e - [Sn]_t) = -kt + \ln ([Sn]_e - [Sn]_0) \qquad \text{(Equation 4.3)}$$

The inorganic tin concentration on Day 5 was used as the equilibrium concentration ($[Sn]_e$). The rate constant was determined by plotting ln ($[Sn]_e$ - $[Sn]_t$) against time (Figure 4.3), where $[Sn]_t$ is the inorganic tin concentration at the time of interest. Biosorption of inorganic tin appearred to be at least a two phase process. The range of the final phase was determined by deleting the earliest data points and performing a linear regression until the correlation coefficient (R^2) was optimized.

The final phase began at *ca.* t = 12 h and continued until the completion of the experiment. Prior to the first 6 h there was rapid uptake of inorganic tin which wasobserved but not measured. The determined rate constant for uptake of tin from estuarine water (t = 12 h to t = 120 h) was 0.010 h^{-1}. Thus, marine algae generally biosorb inorganic tin much faster than *S. alterniflora*. This is probably due to increased surface area on the algae as compared to the marsh grass and to differences in cell wall structure.

In their study of the cycling of methyltin compounds in the presence of *Enteromorpha*, Donard *et al.* (1987) found that high concentrations of Me_3Sn^+ were concurrent with low concentrations of $MeSn^{3+}$ and Me_2Sn^{2+}, and vice versa. Initially they found Me_2Sn^{2+}, but no Me_3Sn^+, in the algal samples. However by day 4 the majority of the MeSn were as Me_3Sn^+. They suggested a detoxification mechanism which ultimately converted the toxic MeSn to volatile Me_4Sn through redistribution reactions (Equations 4.4 - 4.6).

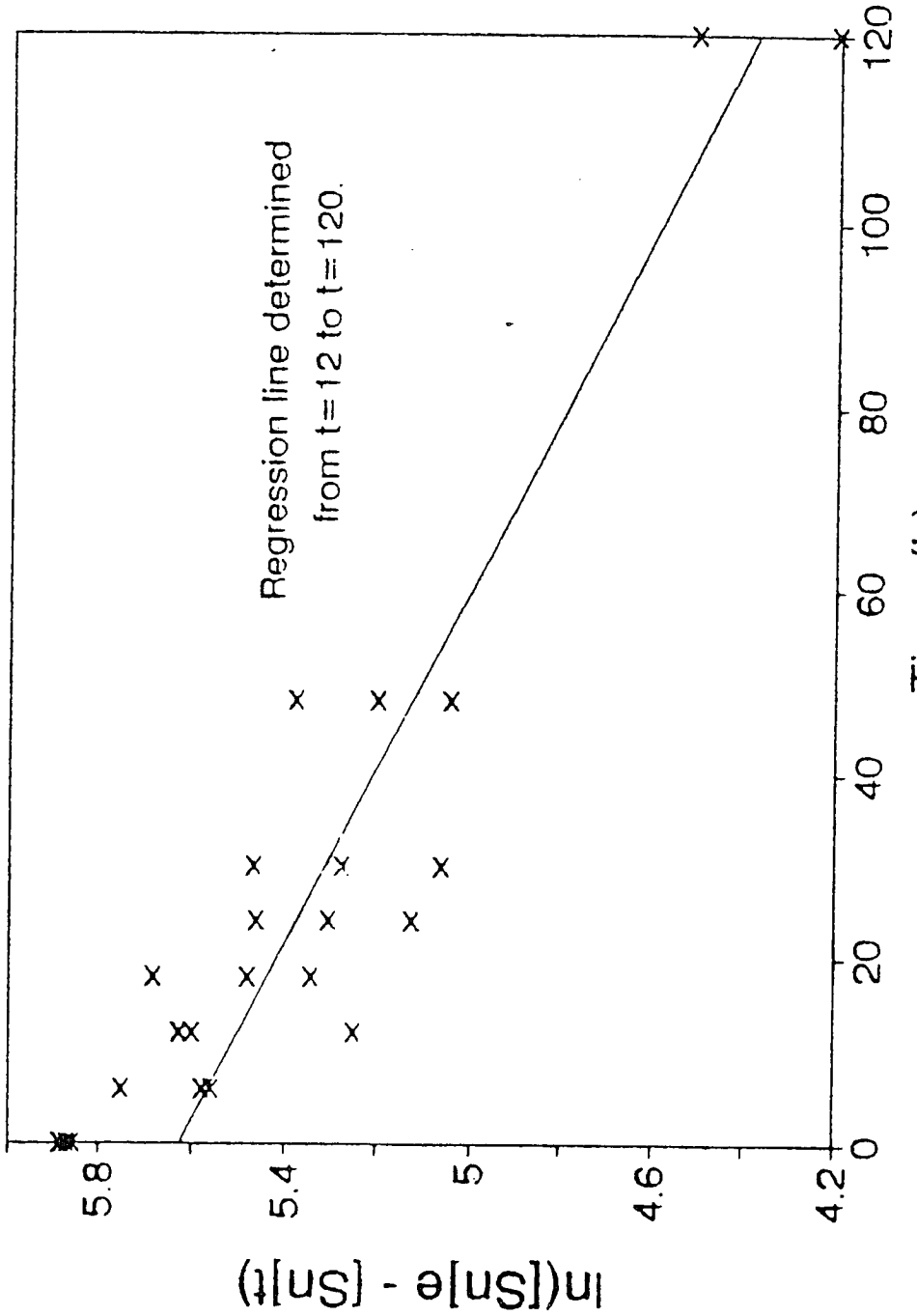

Figure 4.3. Pseudo-first-order kinetic plot for biosorption of inorganic tin by decaying *S. alternifiora* leaves from surrounding estuarine water. Regression line represents biosorption from 12 h to 120 h.

$$4\ Me_3Sn^+ \rightarrow 2\ Me_2Sn^{2+} + 2\ Me_4Sn \qquad \text{(Equation 4.4)}$$

$$2\ Me_2Sn^{2+} \rightarrow MeSn^{3+} + Me_3Sn^+ \qquad \text{(Equation 4.5)}$$

$$3\ Me_3Sn^+ \rightarrow MeSn^{3+} + 2\ Me_4Sn \qquad \text{(Equation 4.6)}$$

We found the same pattern with the Me_2Sn^{2+} and Me_3Sn^+ in the *S. alterniflora* leaves up to Day 5 (Table 4.3). The Me_3Sn^+ concentration increased significantly while the Me_2Sn^{2+} concentration decreased. The increase in Me_3Sn^+ concentration was probably not due to adsorption from the surrounding water as there was never any detectable Me_3Sn^+ in the estuarine water (Table 4.2). This indicates cycling of MeSn. Our study did not continue beyond day 5, so it remains undetermined whether the pattern found by Donard *et al.* (1987) would have been repeated in its entirety. There was no overall change in the concentration of methyl groups bound to tin atoms (Table 4.2) ($F = 1.26$, $df = 8$, $P>F = 0.3319$), so methylation of inorganic tin was unlikely within the time frame studied.

Methylation by Estuarine Water

The total methyl concentration ($[Me]_{tot}$) (Equation 4.1) is an important factor to consider for the understanding of Experiment 2. ANOVA analysis of the variation of $[Me]_{tot}$ with time (Table 4.3) indicated a significant increase in concentration ($F = 8.27$, $df = 2$, $P>F$

= 0.0189). Taken individually, there was significant change in the MeSn concentrations from day to day.

The cycling of MeSn in the estuarine water (Figure 4.3) did not follow the same pattern as that in the leaves (Figure 4.2). In this case, both Me_2Sn^{2+} and Me_3Sn^{+} increased throughout the experiment. In addition, the concentration of $MeSn^{3+}$ decreased on Day 2 and then returned to the original concentration on Day 4. This is consistent with the following methylation and redistribution reactions (Equations 4.7 - 4.10):

$$5\ Sn + 5\ Me \rightarrow 5\ MeSn^{3+} \qquad \text{(Equation 4.7)}$$

$$4\ MeSn^{3+} \rightarrow 2\ Me_2Sn^{2+} + 2\ Sn^{4+} \qquad \text{(Equation 4.8)}$$

$$MeSn^{3+} + Me_2Sn^{2+} \rightarrow Me_3Sn^{+} + Sn^{4+} \qquad \text{(Equation 4.9)}$$

$$5\ Sn + 5\ Me \rightarrow Me_2Sn^{2+} + Me_3Sn^{+} + 3\ Sn^{4+} \qquad \text{(Equation 4.10)}$$

'Me' is used to represent a methyl group. It may be a methyl cation, methyl radical, or a methyl anion. In Equations 4.7 and 4.10 inorganic tin is expressed without an oxidation state because, in the case of environmental tin, the oxidation state is unknown. Methylation may be by oxidative addition of a methyl cation to Sn(II). This readily occurs in model systems using methyl iodide as the methyl donor (Lee and Weber, 1988).

$$Sn(II)^{2+} + MeI \rightleftharpoons MeSn^{3+} + I^{-} \qquad \text{(Equation 4.11)}$$

Further methylation to Me_2Sn^{2+} or Me_3Sn^+ via a carbocation donor is unlikely since it would first require a reduction step to unknown MeSn(II) analogs. Thus, the suggested rearrangement reactions are more likely.

Conclusions

Apparently, two separate processes account for the changes in MeSn concentrations in *S. alterniflora* leaves (Experiment 1) and in the water (Experiment 2) since there were two distinctly different trends. There was no net methylation in the leaves (Experiment 1) however, there was noticible rearrangement from Me_2Sn^{2+} to Me_3Sn^+. In MeSn amended water alone (Experiment 2) there was an increase in concentrations of both Me_2Sn^{2+} and Me_3Sn^+ accompanied by an overall net methylation. The pathway to MeSn in estuarine systems remains uncertain. *S. alterniflora* leaves absorb inorganic tin (and probably MeSn) from the surrounding water. Then it appears that MeSn in the leaves were rearranged predominately forming Me_3Sn^+. Decaying *S. alterniflora* comprise a large portion of the organic litter in the estuary each year. This addition to the organic matter found at the surface of the sediment may contribute to the MeSn found regularly in healthy *S. alterniflora.*

CHAPTER V

SPECIATION OF METHYLTIN COMPOUNDS BY GAS CHROMATOGRAPHIC MASS SPECTROMETRY

Introduction

Researchers have used many techniques for speciation of methyltin compounds (MeSn) following volatilization by hydride generation, including gas chromatography (GC) connected with atomic emission spectroscopy (Braman and Tompkins, 1979; Anderae and Byrd, 1984), atomic absorption spectrometry (AAS) (Donard *et al.*, 1986), and mass spectrometry (MS) (Gilmour *et al.*, 1985). The marriage of GC and AAS provides a sensitive and selective means for MeSn speciation, however, compound identity is based solely on retention time. While often adequate, this does not provide definitive identification which is often necessary. GC-MS inherently has 2 separation axes. The first, provided by the GC, is based on volatility, polarity, or size of the analyte molecules. The second, provided by the MS, is based on mass of the molecular fragments.

GC-MS has been used for the sensitive and definitive determination of MeSn (Means and Hulebak, 1983; Gilmour *et al.*, 1986). Ashby and Craig (1991b) used GC-AAS following ethylation by sodium tetraethylborate to analyze a wide range of organotin compounds. They

confirmed the identity of the ethylated derivatives using GC-MS. However, since GC-MS is both a quantitative and a qualitative tool, with the proper set-up both the analysis and the identification can be performed in one step. Gilmour *et al.* (1986) determined MeSn in sediment samples using a packed column GC coupled with a MS. They purged volatile MeSn hydrides along with diethyltin dihydride internal standard directly onto the head of a packed GC column held at -40°C. Using selective ion monitoring at the MS, they obtained detection limits down to 5 pg with a linear range from 0.1 to 15 ng and correlation coefficients for calibration curves greater than 0.99. Other researchers have used essentially the same method to determine MeSn in kidney and brain tissues (Means and Hulebak, 1983).

The true advantage of GC-MS as a tool for the study of environmental methylation of tin lies not in its use as a sensitive, selective detector, but in its ability to discern between isotopes of the same element. The advantage to this is two-fold. (1) By way of isotope dilution, deuterated homologues or another isotope of the analyte may function as internal standards. Physicochemical properties of both the internal standard and the analyte are virtually identical, providing optimal compensation for analyte losses at all steps of the analytical process. In their work with butyl- and phenyl-tin compounds, Kon *et al.* (1991) found improved recovery and analytical precision using an isotopically labeled homologue of the analyte as an internal standard rather than a compound similar to the analyte(s). (2) An isotope of tin not commonly found in the environment may be used as a tracer for environmental mechanisms. If an uncommon isotope of the analyte is doped into an environmental system, it can be traced through all mechanisms of the system. The two-fold

separation capabilities of the GC-MS make tracer studies using isotopic tracers an ideal way to capitalize on the advantages of this instrumentation. Criag and Rapsomanikis (1984) were among the first to truly capitalize on the advantages of the GC-MS for tin methylation studies. They sought to determine the origin of Me_4Sn in environmental systems. To do this they incubated estuarine and freshwater sediments with $(CD_3)_3SnCl$. Production of $(CD_3)_3MeSn$ would indicate environmental methylation, while production of $(CD_3)_4Sn$ would indicate rearrangement of the initial $(CD_3)_3SnCl$. Injection of headspace gases from the experiment into capillary column GC coupled with a MS allowed distinction between $(CD_3)_3MeSn$ and $(CD_3)_4Sn$.

Craig and Rapsomanikis (1984) used MS for distinction between volatile MeSn products. There was no need for derivatization of other MeSn products, and therefore, no need to couple a hydride generation process with the capillary column GC. Other researchers (Gilmour *et al.*, 1986; Seligman *et al.*, 1988) coupled the hydride generator to a packed column GC because of its greater capacity as compared to capillary column GC. Use of capillary columns for separation of the volatile MeSn components would improve both resolution and sensitivity. However, the shift to a capillary column requires an interface capable of overcoming the high gas volumes generated by the hydride generation step. Commonly, a splitter is used for this purpose which essentially discards 90% of the sample, considerably reducing the method sensitivity. In this chapter I examine the use of both cryotrapping and isothermal trapping coupled with ballistic heat desorption with low flow rates as a means to overcome the incompatability of hydride generation interfaced with

capillary column GC. Additionally, in order to improve peak shape and hence, sensitivity and precision, cryofocussing at the head of the capillary column was employed. This work provides the background necessary for sensitive and selective speciation of MeSn enabling methylation studies using an uncommon isotope of tin as a tracer.

This is work in progress. Many experimental parameters have been explored which point the direction for future work, however, there are still problems with the method that must be addressed before it can be used as a reliable research tool.

Experimental

Materials

All glassware and plasticware was acid cleaned in 10% HNO_3 for at least 48 h then rinsed in distilled, deionized water. Water used in all experiments was doubly deionized and distilled through a Corning Mega-pure still. This water was periodically tested for inorganic tin and MeSn and found to be free of these compounds within the detection limits of the system (10 pg ml^{-1}). $MeSn^{3+}$, Me_2Sn^{2+}, and trimethyltin (Me_3Sn^{+}) chlorides are of 97+% purity (Alpha Chemicals). Stock solutions of *ca.* 1000 mg ml^{-1} (all concentrations as Sn) were prepared in 1 M HCl. Standards were prepared weekly by diluting the stock solutions to

ca. 5 μg ml^{-1} in 0.05 M HCl. A 6% solution of sodium borohydride ($NaBH_4$) was prepared by dissolving 12 g $NaBH_4$ in 100 ml H_2O, stirring and allowing to sit, covered, in a refrigerator overnight. The solution was then filtered through a 0.2 μm polycarbonate filter (Nuclepore) to remove any Sn colloids, and diluted to 200 ml with H_2O. All Tenax products were from Alltech. Deuterated methyliodide (CD_3I) (95% purity) was obtained from Aldrich Chemicals.

Preparation of CD_3SnI_3 Internal Standard

Deuterated monomethyltin iodide (CD_3SnI_3) was prepared using a method developed by Kennedy (1975). A solution of potassium hydroxide (3.3 g in 13 ml H_2O) at 0°C was added to a solution of stannous chloride (1.4 g in 3.0 ml H_2O) (also at 0°C) forming potassium stannite. A cooled solution of deuterated methyl iodide (1.02 g dissolved in 3.0 ml absolute ethanol) was added to the SnCl solution with 3 x 1 ml ethanol rinses and the solution was stirred overnight. Addition of small pieces of solid carbon dioxide under a CO_2 atmosphere for 2 h results in a white crystalline precipitate. After the volatile components were removed under reduced pressure water (10 ml), carbon tetrachloride (10 ml), and enough hydroiodic acid to dissolve the solid were added. The organic layer was separated and the aqueous layer was then extracted several times with CCl_4. Evaporation of CCl_4 left behind the crude CD_3SnI_3 which was purified by recrystallizing in light petroleum ether. The identity of the crystals was confirmed by both carbon and deuterium NMR and GC-MS.

Instrumentation

The overall system used consists of a hydride generation vessel, a means of trapping the hydrides while allowing other gasses to be vented to the atmosphere, a means of removing water vapor before it enters the capillary column, a chromatographic column for separation of compounds and a detector (Figure 5.1). In this case a sealed 30 ml serum vial was used as the hydride generator. An 18 gauge needle connected to 1/8 in o.d. Teflon tubing via a luer connector was inserted just through the septum. Helium flow at 10 ml min^{-1} was directed into the vial via a second needle (22 gauge) inserted through the septa and under the surface of the solution. The other end of the tubing was connected at position 1 to a 6-way valve, which was connected at positions 2 and 5 to the cryotrap. The trap was composed of a short length (15 cm) of 1/8 in o.d. Teflon tubing packed at the middle 5 cm with 3% SP-2100 on Supelcoport 60/80 mesh. The packing material was held in place with a small plug (*ca.* 1 cm) of silanized quartz wool on either end. The trap and 6-way valve were set up so that as the sample was purging onto the trap, the excess H_2 and He pass through the trap and out into the atmosphere (at position 6). Teflon tubing (1/8 in o.d.) connected a helium source through a flow meter/regulator (Cole-Parmer) and to position 4 of the 6-way valve. The flowmeter regulates the He flow to 1 ml min^{-1}. When the valve was switched, He flowed through the trap in reverse of the direction of the loading phase and out at position 3 to the GC. The connection to the GC is made through a 23 gauge needle connected via a luer connector to Teflon tubing. The needle was inserted into the injection port of the GC. A silanized double gooseneck sleeve was used in the injection port. Hydrides were focussed at the head of the

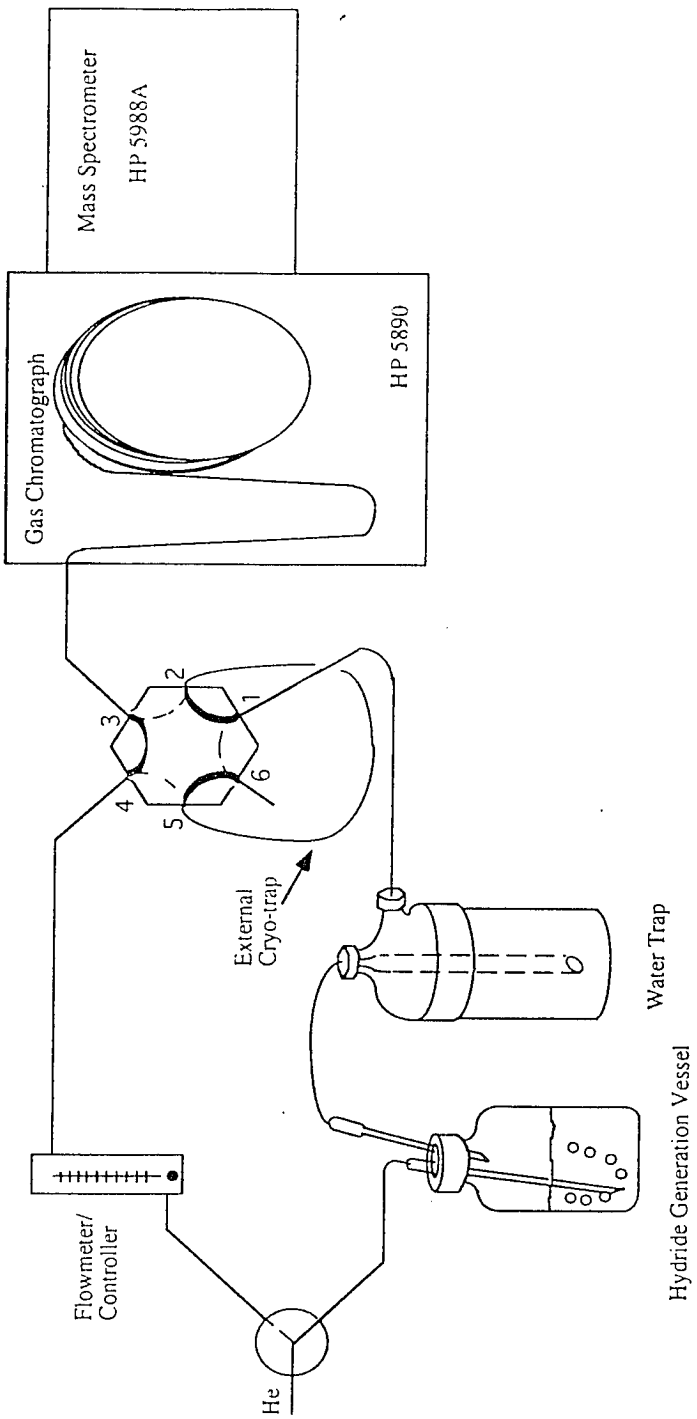

Figure 5.1. Schemata of the hydride generation-GC-MS set-up. On the valves, solid lines indicate the valve position during the loading phase of the analysis. The dashed lines indicate the position used to purge the analytes from the external trap onto the GC-MS.

capillary column by immersing the first loop of the column in liquid nitrogen. Separation of hydrides was achieved on a 50 m capillary column (HP-1 with 0.2 mm i.d. and 0.5 μm dimethylsiloxne film) by gas chromatography (Hewlett Packard 5890) (Furnace program: T_i = 30°C, ramp = 10°C min^{-1}, T_f = 120°C, t = 5.00 min). The GC was linked to a Hewlett Packard Quadrupole Mass Spectrometer (HP 5988A) (Electron impact, 70 eV; selective ion monitoring (SIM) acquisition, monitoring ions m/z: 133, 135, 140, and 151).

Determination of Inorganic Tin and Methyltin Compounds

MeSn and inorganic tin standards (2 - 25 ng as Sn), along with 5 ml of 5 mg ml^{-1} CD_3SnI_3 were added to a 30 ml serum vial containing 20 ml 0.05 M HNO_3. The serum vial was capped with a Teflon lined septum and a crimp-on seal (Supelco). To generate hydrides, 1 ml of 6% $NaBH_4$ was added via a syringe through the septum and the sample was agitated (VWR Vortexor II) at low speed for 10 min. Samples were analyzed within 4 h of hydride generation to obtain quantitative results.

To analyze the MeSn content of samples, the cryo-trap was first placed into a dewar of liquid nitrogen. A large (18 gauge) outlet needle was inserted through the septa of the serumvial. Care was taken to ensure that the opening of the needle is through the septum and not into the solution, and that the needle was not plugged with septum material. A second

needle, connected to the helium flow was inserted through the septum and into the solution. The three-way valve was switched so that helium flows through the solution, purging hydrides through the larger outlet needle for 7 min. Volatile hydrides were carried on the helium stream until they were trapped on the liquid nitrogen-cooled trap. Excess hydrogen passed on through the trap and into the atmosphere.

An alternative trapping mechanism was also employed. A micro column consisting of a 10 cm length of 1/8 in i.d. quartz tubing wrapped with nichrome wire (28 gauge, 65 Ω resistance) and packed at the middle 4 cm with Tenax-GC held in place with a 1 cm plug of silanized quartz wool at both ends was connected to a large bore (18 gauge) needle. The needle was inserted just through the septum of the hydride generation vial. After trapping the analytes for 7 min, the trap was disconnected from the vial, connected to the helium flow and inserted in the injection port of the GC. Analytes were thermally desorbed by passing a current through the nichrome wire, heating the column to 200°C.

The next phase in the analytical process involved desorption of the analytes from the cryotrap and focusing them at the head of the analytical column. To focus the analytes at the head of the analytical column, the first loop of the capillary column was immersed in a dewar of liquid nitrogen. Then, so that there was no back-flow of the liquid sample, the needle carrying the helium flow was removed from the solution. The three-way valve was switched to position 2, directing the helium through the flowmeter and into the 6-way valve. The 6-way valve was switched to position 2, directing the helium through the cryo-trap in reverse of

the direction the sample was collected. The analytes were purged from the cryotrap onto the head of the GC column for 2 min. Upon removal of liquid nitrogen the GC program and the MS program were started. MeSn eluted in order of their boiling points and were detected by the MS.

Data acquisition at the MS was set up as specified in Table 5.1. Quantitation may be based on either comparison of the chromatographic peak area to a standard curve or comparison of the ion intensities of selected ion peaks with that selected for CD_3Sn^+.

Results and Discussion

Characterization of Internal Standard

To confirm the presence and purity of CD_3SnI_3 internal standard, it was checked by carbon NMR (Figure 5.2), deuterium NMR (Figure 5.3), and GC/MS (Figure 5.4). The carbon NMR scan showed a septuplet, indicating a carbon signal split by 3 deuterium atoms. Additionally, the deuterium NMR showed a strong deuterium interference pattern, indicating, again, that the major product was CD_3SnI_3. The isotope scan of CD_3SnI_3 (Figure 5.4a) compared to that of $MeSn^{3+}$ (Figure 5.4b) showed an m/z shift 3 units heavier. All of

Table 5.1. Operating parameters for the gas chromatographic mass spectrometer.

Gas Chromatograph	Initial Temperature (T_i)	30°C
	Initial Time	0 s
	Ramp rate	10°C min^{-1}
	Final Temperature (T_f)	120°C
	Time	5.0 min
Mass Spectrometer	Splitless valve off	0.25 min
SIM aquisition	**Analyte**	**m/z (time)**
	$MeSn^{3+}$	133 (1.2 - 1.5 min)
	CD_3Sn	135 (1.2 - 1.5 min)
	Me_2Sn^{2+}	140 (1.5 - 1.9 min)
	Me_3Sn^{+}	151 (1.9 - 5.0 min)

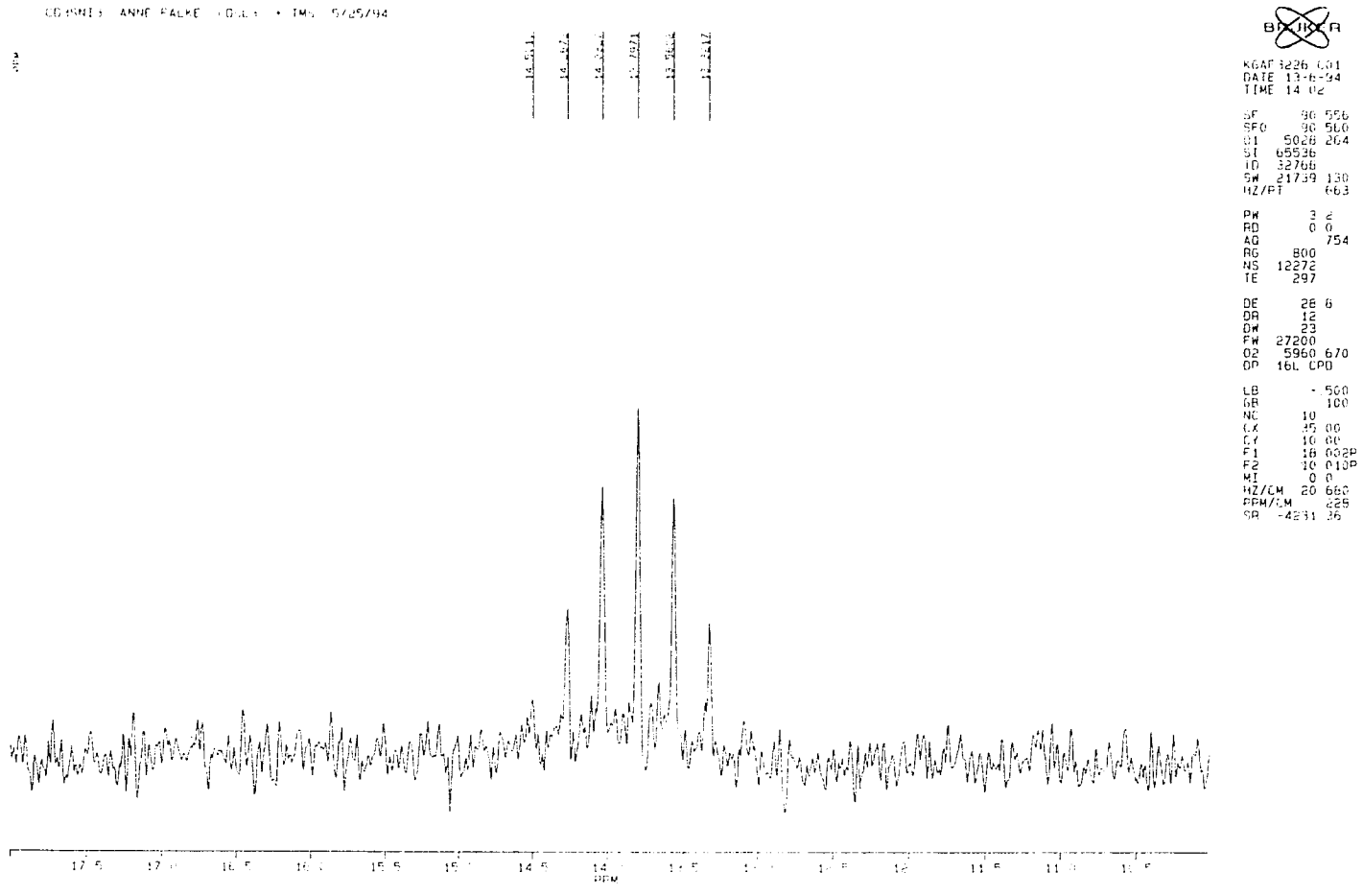

Figure 5.2. Carbon NMR of CD_3SnI_3.

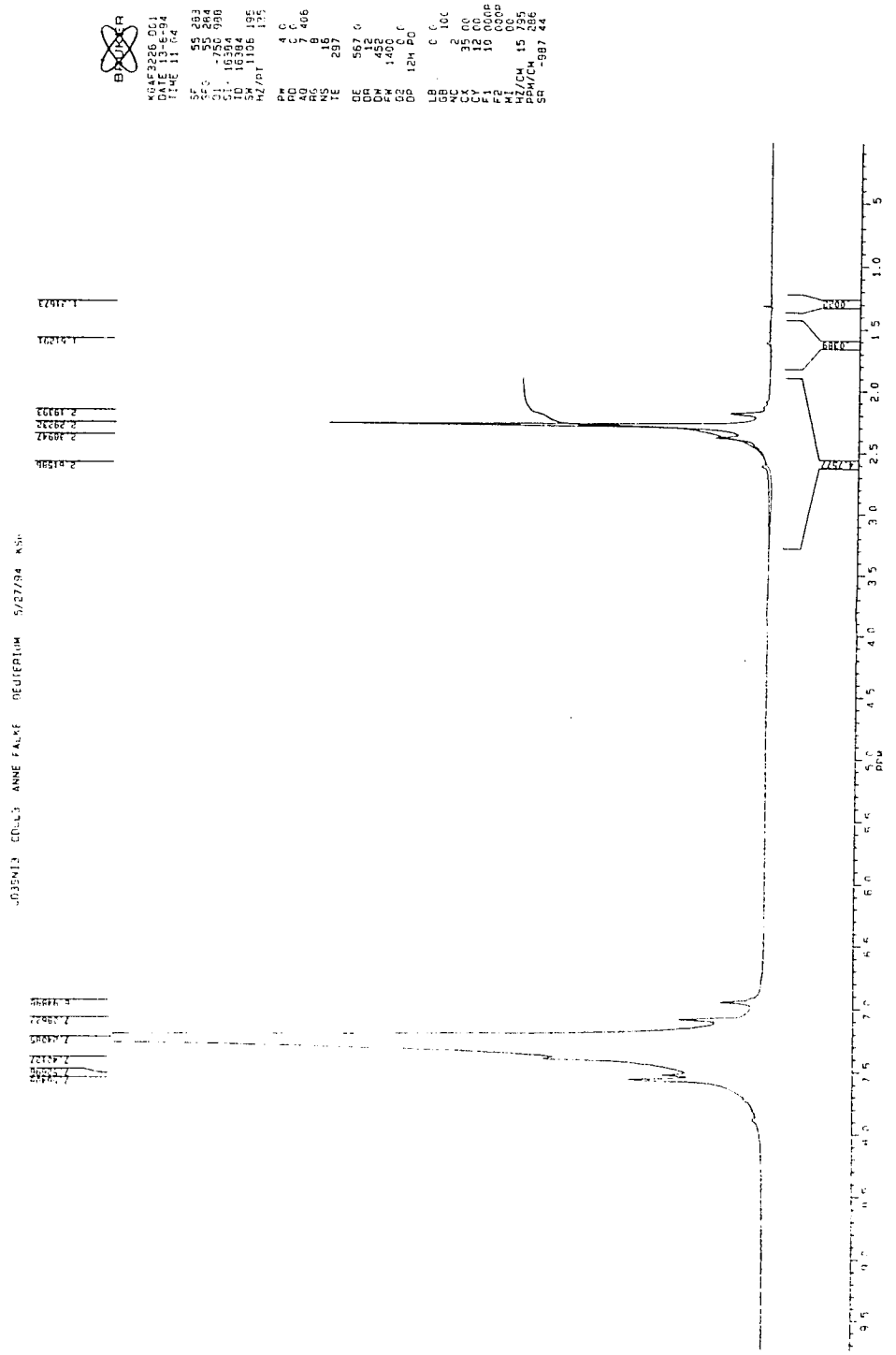

Figure 5.3. Deuterium NMR of CD_3SnI_3.

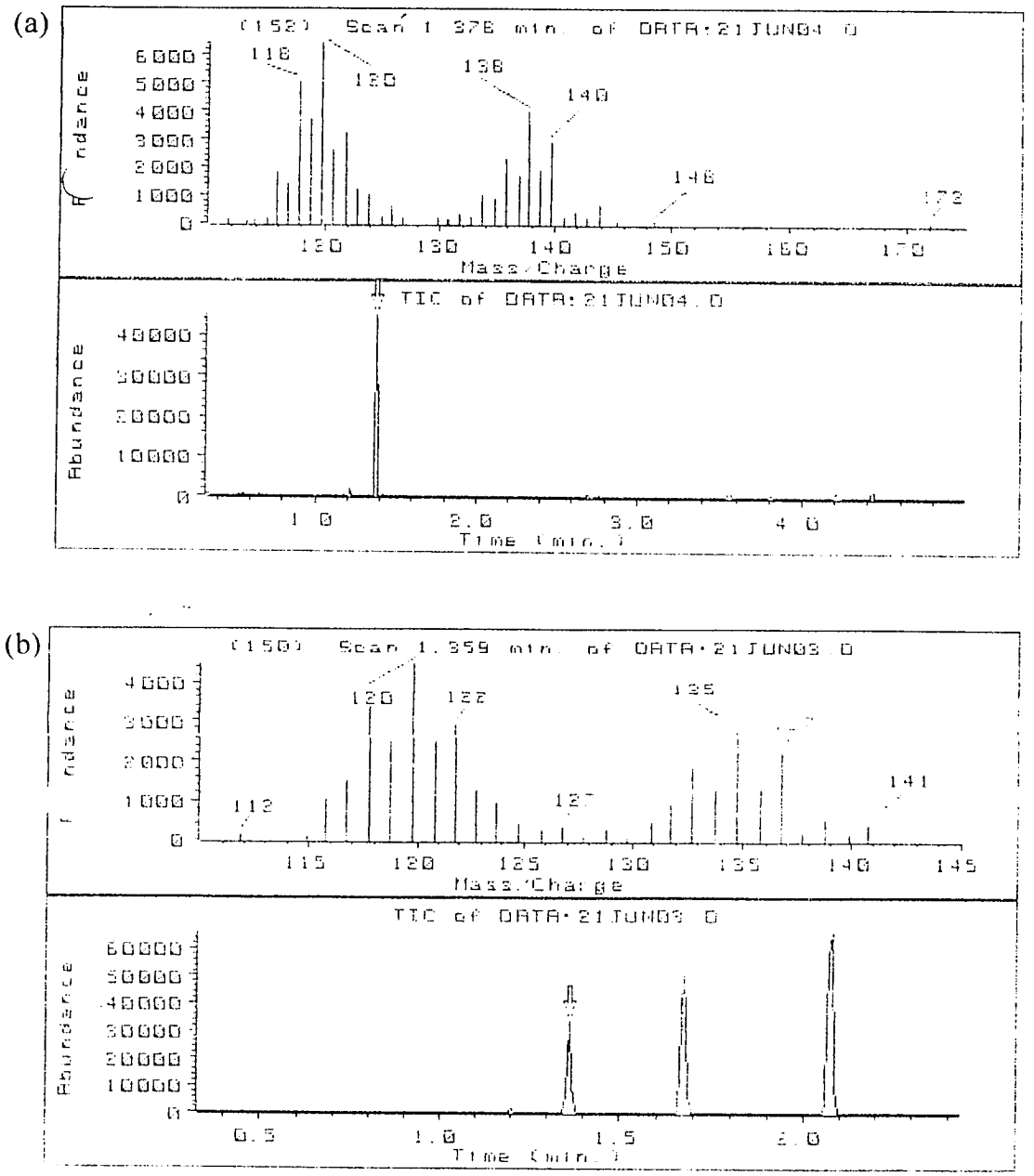

Figure 5.4. Total ion chromatograph (TIC) and isotope scan of (a) CD_3SnI_3 and (b) $MeSn^{3+}$ (TIC also includes Me_2Sn^{2+} and Me_3Sn^{+}).

these indicated that the major product was CD_3SnI_3 and that $MeSn^{3+}$ represented only a minor component (<5%) of the internal standard.

External Trapping

Two different methods were used to trap MeSn hydrides prior to introducing them to the GC/MS: cryotrapping in a packed micro column immersed in liquid nitrogen and isothermal trapping on a short Tenax-GC column. Calibration curves obtained using each of these methods are shown in Figures 5.5 and 5.6. Slopes and correlation coefficients for the curves are compared in Table 5.2. Slopes for the three MeSn compounds by the cryotrapping method were comparable, indicating comparable trapping and release efficiencies for each of the analytes. This was not true of the Tenax trapping method. For the analysis used for the calibration curve, the efficiency of getting $MeSn^{3+}$ to the detector was less then 10% of that for Me_3Sn^+. However, traps were variable. On another day, with a different trap, precision data were gathered. The sensitivity for all of the MeSn compounds was greater and there was not as much discrepancy between each of the analytes. The probable source of the difference in trapping/release efficiencies was probably connected to the column conditioning. If the column was incompletely conditioned, active binding sites may still exist, and the more polar $MeSn^{3+}$ would not all desorb from the Tenax upon heating. Correlation coefficients for the calibration curves of each of the analytes determined by the cryotrapping method indicated

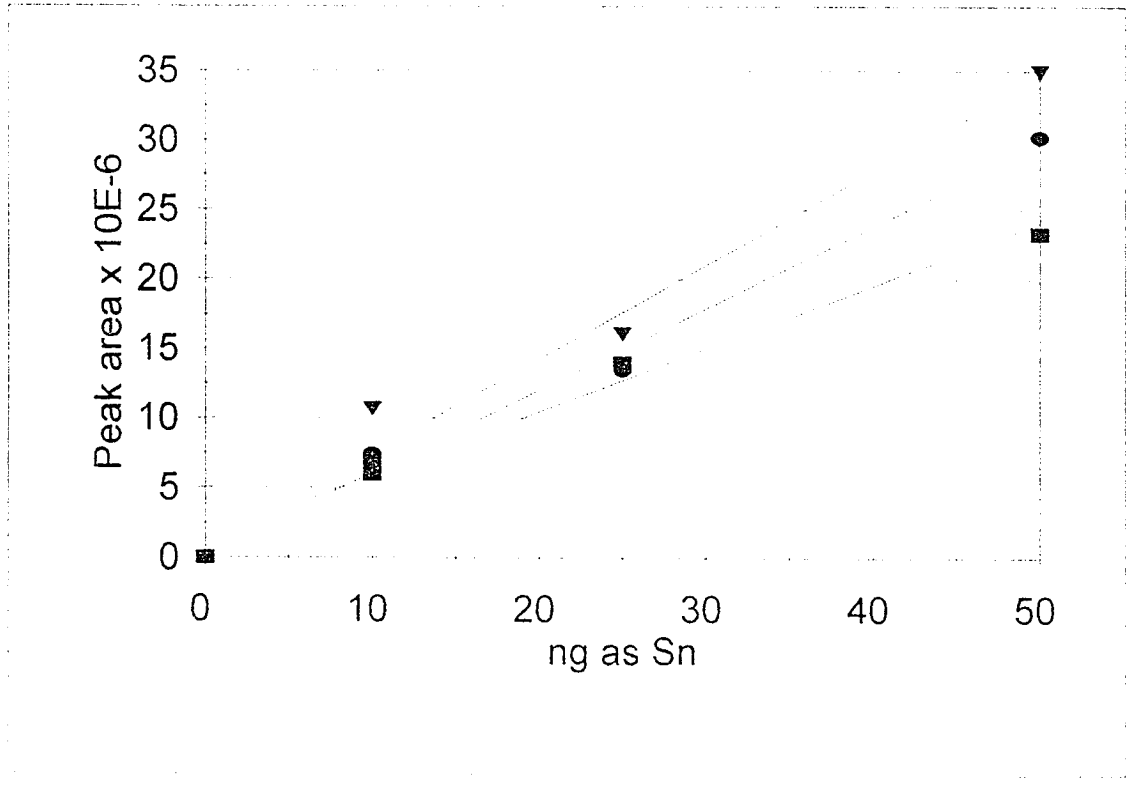

Figure 5.5. Calibration of monomethyltin (■), dimethyltin (●), and trimethyltin (▼) standards using cryotrapping in liquid nitrogen to concentrate analytes prior to analysis.

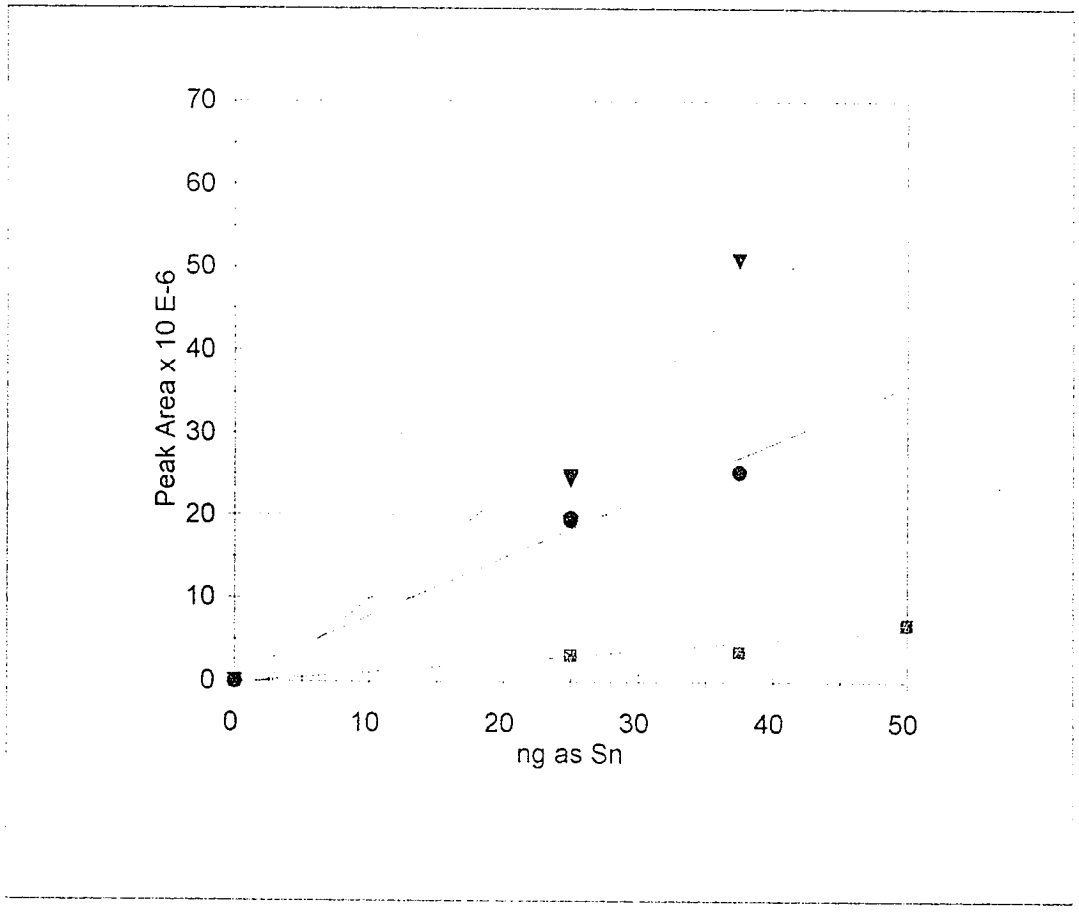

Figure 5.6. Calibration of monomethyltin (▣), dimethyltin (●), and trimethyltin (▼) standards using a Tenax-GC trap to concentrate analytes prior to analysis.

Table 5.2. Calibration and precision data for GC-MS analysis of methyltin compounds comparing externally trapping compounds at liquid nitrogen temperatures to trapping on Tenax-GC.

		Slope	R^2	Mean[a] (peak area x 10^{-6})	RSD[a]
$MeSn^{3+}$	Cryotrap	0.494	0.952	4.416	4.7%
	Tenax-GC	0.123	0.930	20.36	5.5%
Me_2Sn^{2+}	Cryotrap	0.597	0.989	4.90	6.4%
	Tenax-GC	1.078	0.765	72.5	5.7%
Me_3Sn^{+}	Cryotrap	0.697	0.967	5.52	3.9%
	Tenax-GC	1.722	0.781	71.6	16%

[a]Mathematical average and relative standard deviation (RSD) for 3 consecutive analyses of MeSn (25 ng each as Sn). The precision samples for the samples trapped on Tenax were analyzed using a different trap than the calibration samples.

acceptable curves. However, curves determined for Me_2Sn^{2+} and Me_3Sn^{+} using Tenax trapping were not linear enough to be analytically useful.

Breakthrough volumes. Two Tenax columns were connected in series to the hydride generation flask. The degree of breakthrough was determined by separately desorbing each column and running the GC-MS program after hydride generation with 25 ng and 50 ng of MeSn. Comparison of the second column to the first indicated the percentage of analytes breaking through the trap. No MeSn were detected on the second trap. A similar analysis was made using the cryotrap. Two packed columns were connected in series and both were immersed in liquid nitrogen. The second trap remained in liquid nitrogen while the first was analyzed. For up to 50 ng of each of the MeSn's, no breakthrough was detected.

Carryover. To determine carryover using the cryotrapping method, a high standard was analyzed followed immediately by analysis of a blank using the same trap for both. Analysis of a blank following analysis of 25 ng of each of the MeSn compoundsshowed no detectable levels of MeSn.

To determine carryover for the Tenax trap, immediately following analysis of a high standard (50 ng MeSn) the trap was analyzed a second time by connecting it to the GC/MS and ballistically heating it to 200°C. No MeSn were detected in the second analysis of the trap.

Rearrangement. Each MeSn compound was analyzed individually to see that the compounds of interest were not redistributing in the cryotrap or during the desorption phase. For each analysis of the 3 MeSn, only the spiked MeSn was detected. This test was not conducted using the Tenax trap.

Water Removal

Peak shapes were significantly improved by cryofocussing at the head of the analytical column. However, immersing a narrow bore column in liquid nitrogen and introducing a gaseous sample that was produced by hydride generation in aquous media creates additional problems. Any water that gets into the column freezes and creates blockages. Water removal was, necessarily, a crucial step. A Teflon midget impinger in solid CO_2 removed most of the water but was not entirely effective. After about 4 or 5 analyses, the sensitivity of the system was reduced to almost nothing. Heating the column to 120°C for an hour often improved the sensitivity to almost normal. Chemical desiccants ($CaSO_4$, silica, and molecular sieves) were unsatisfactory as driers as the analyte would stick to the desiccant. A dryer (Perma Pure, Inc., Toms River, NJ) consisting of an inner tube made of Nafion which is permeable to water surrounded by an outer tube with a concurrent flow of dry gas was also tried. While this was somewhat promising, there were still problems detecting analytes every few runs. This may be due to the small amount of water getting through the system or to small, undetected leaks. This problem is, as of now, unsolved and should be addressed in depth before continuing with the method development.

Calibration

A typical chromatogram of inorganic tin and MeSn standards (no internal standard) is shown in Figure 5.7. This particular analysis used an external trap consisting of a short loop of packed Teflon tubing immersed in liquid nitrogen. All compounds were well separated and peaks were narrow and symmetrical.

Calculation of analyte concentration ([A]) by comparison to CD_3SnI_3 internal standard ([IS]) was based on work by Colby *et al.* (1981):

$$[A] = \frac{(R_{IS} - R_M)(R_A + 1)[IS]}{(R_M - R_A)(R_{IS} + 1)} \qquad \text{(Equation 5.1)}$$

where R_A is the ion intensity ratio of the analyte at the *m/z*'s chosen, R_{IS} is the ion intensity ratio of the internal standard at the same *m/z*'s, and R_M is the ion intensity ratio of a mixture of analyte and internal standard at those *m/z*'s. This equation takes into account interference patterns created by contribution to the *m/z* chosen for the internal standard by the analyte and contribution to the *m/z* chosen for the analyte by the internal standard. To gain the maximum benefit from the use of isotope dilution MS, the ion peaks used should be chosen so that R_A approaches infinity and R_{IS} approaches zero. If $10\ R_{IS} < R_M < 0.1\ R_A$ then Equation 5.1 reduces to:

$$[A] = R_M[IS] \qquad \text{(Equation 5.2)}$$

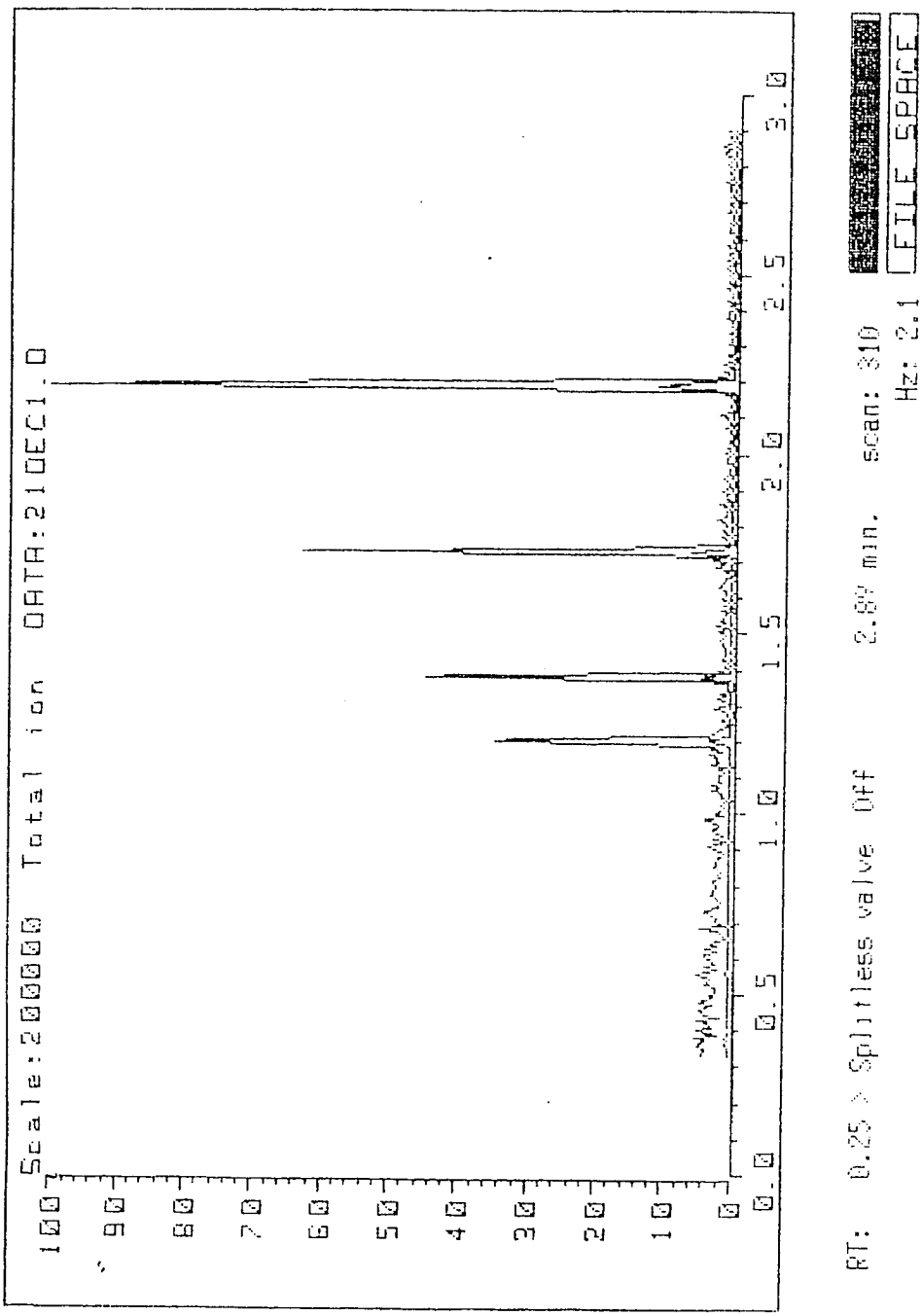

Figure 5.7. A typical chromatogram of inorganic tin (1.20 min), $MeSn^{3+}$ (1.38 min), Me_2Sn^{2+} (1.73 min), and Me_3Sn^{+} (2.19 min) obtained by hydride generation, cryogenic trapping, gas chromatographic separation,and detection by mass spectrometry.

Figure 5.8 shows actual $MeSn^{3+}$ concentration compared to that determined from isotope dilution MS using equation 5.2. This curve demonstrates reasonable agreement of experimentally determined amounts of $MeSn^{3+}$ to actual amounts (R^2 = 0.955). Figure 5.9 shows the comparison of theoretical Me_2Sn^{2+} and Me_3Sn^+ amounts based on comparison to a CD_3Sn^{3+} internal standard. Apparently, Me_2Sn^{2+} and Me_3Sn^+ behaved very differently from $MeSn^{3+}$ in the analysis. In order to adequately determine Me_2Sn^{2+} and Me_3Sn^+ using isotope dilution, deuterated analogues of these compounds should be prepared and used. Given the variability of the system, isotope dilution was probably much more accurate than comparing sample concentrations to a calibration curve.

Conclusions

The usefulness and possibility of a hydride generation/gas chromatographic mass spectrometry method capitalizing on the high separation efficiency of capillary column GC has been demonstrated by these experiments. Use of external trapping and cryofocussing with HG/GC-MS greatly enhances peak shape and size and, therefore, sensitivity and precision of MeSn analysis by GC-MS. It appears, from these experiments, that the most reliable, accurate means of external trapping was cryofocussing in liquid nitrogen. Isothermal trapping on Tenax does have the advantages of simplicity and increased speed (several samples can be collected simultaneously and maintained on the traps until analyzed), so it is worth further effort. The use of deuterated homologues as internal standards improves the

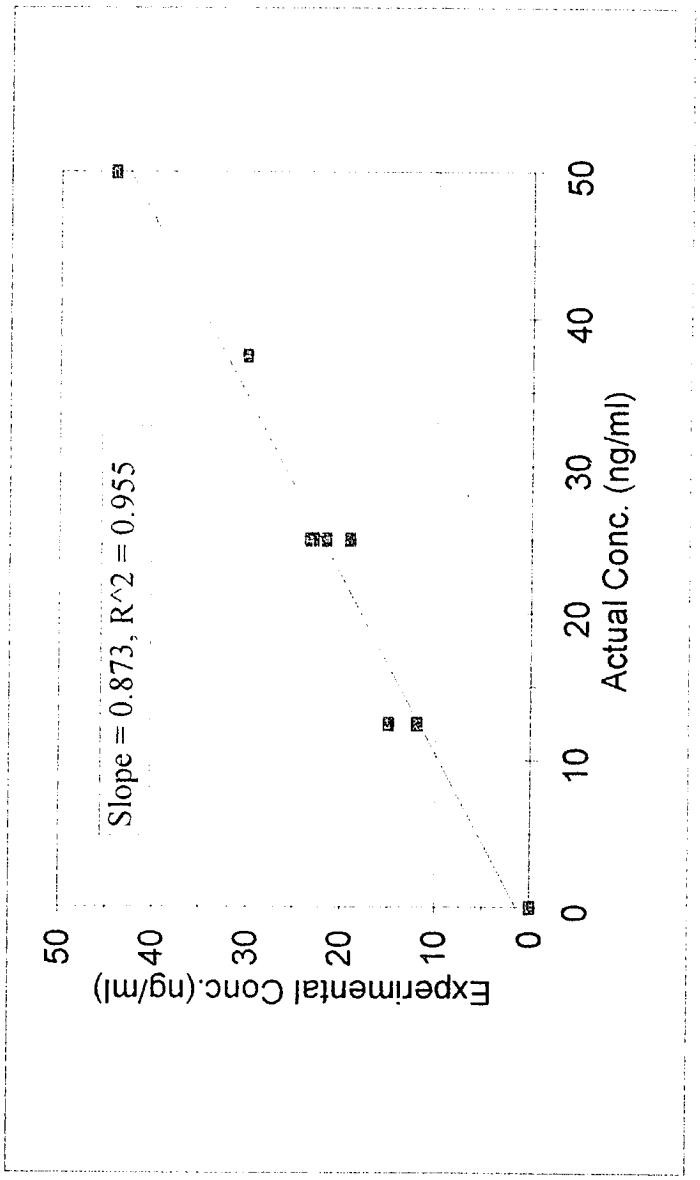

Figure 5.8. Correlation of monomethyltin concentrations determined by isotope dilution mass spectrometry to actula concentrations.

Figure 5.9. Correlation of dimethyltin (●) and trimethyltin (■) concentrations determined by isotope dilution mass spectrometry to actual concentrations.

method precision over a wide range of concentrations, however, it was useful only for the homologue analyte. In order to be truly useful, deuterated homologues of each of the analytes must be used. Intermittent problems still persist with the method. In spite of all efforts to remove water, it still seems to have a deleterious effect on the column and detector. Additionally, CO_2 seems to be leaking into the system and causing sporadic problems. With a little more work, these problems can be overcome and HG/GC-MS determination of MeSn will be a viable method.

LIST OF REFERENCES

Alberts, J. J.; Price, M. T.; Kania, M., *Est. Coast. Shelf Sci.*, **1990**, *30*, 47-58.

Anderae, M. O.; Byrd, J. T., *Anal. Chim. Acta*, **1984**, *156*, 147-157.

Anagnostopoulos, A.; Hadjispyrou, S., *Toxicol. And Environm. Chem.*, **1994**, *41*, 175-186.

Ashby, J. R.; Craig, P. J., *Sci. Tot. Environ.*, **1991a**, *100*, 337-346.

Ashby, J. R.; Craig, P. J., *Appl. Organomet. Chem.*, **1991b**, *5*, 173-181.

Ashby, J. R.; Craig, P. J., *Sci. Tot. Environ.*, **1988**, *73*, 127-133.

Bloom, N.; Effler, S. W., *Wat. Air Soil Pollut.*, **1990**, *53*, 251-265.

Braman, R. S.; Tompkins, M. A., *Anal. Chem.*, **1979**, *51*, 12-19.

Burns, D. T.; Harriott, M.; Glockling, F., *Fres. Z. Anal. Chem.*, **1987**, *327*, 701-703.

Burns, D. T.; Glockling, F.; Harriott, M., *Analyst*, **1981**, *106*, 921-930.

Chau, Y. K.; Wong, P. T. S., *Fres. J. Anal. Chem.*, **1991**, *339*, 640-645.

Chau, Y. K.; Wong, P. T. S.; Bengert, G. A.; Wasslen, J., *Appl. Organomet. Chem.*, **1988**, *2*, 427-433.

Chau, Y. K.; Wong, P. T. S.; Bengert, G. A., *Anal. Chem.*, **1982**, *54*, 246-249.

Colby, B. N.; Rosecrance, A. E.; Colby, M. E., *Anal. Chem.*, **1981**, *53*, 1907-1911.

Craig, P. J.; Rapsomanikis, S., *Environ. Technol. Lett.*, **1984**, *5*, 407-416.

Craig, P. J.; Rapsomanikis, S., *J. Chem. Soc. Chem. Commun.*, **1982**, 114.

Dacey, J. W. H.; King, G. M.; Wakeham, S. G., *Nature*, **1987**, *330*, 643-645.

Dizikes, L. J.; Ridley, W. P.; Wood, J. M., *J. Am. Chem. Soc.*, **1978**, *100*, 1010-1012.

Donard, O. F. X.; Quevauviller, P.; Bruchet, A., *Water Res.*, **1993**, *27*, 1085-1089.

Donard, O. F. X.; Weber, J. H., *Nature*, **1988**, *332*, 339-341.

Donard, O. F. X.; Short, F. T.; Weber, J. H., *Ca.n. J. Fish. Aquat. Sci.*, **1987**, *44*, 140-5.

Donard, O. F. X.; Rapsomanikis, S.; Weber, J. H., *Anal. Chem.*, **1986**, *58*, 772-777.

Ebdon, L.; Hill, S. J.; Jones, P., *Analyst*, **1985**, *110*, 515-517.

Errecalde, O.; Astruc, M.; Maury, G.; Pinel, R., *Appl. Organomet. Chem.*, **1995**, 23-28.

Falke, A. M.; Weber, J. H., *Appl. Organomet. Chem.*, **1994,** *8*, 351-359.

Falke, A. M.; Weber, J. H., *Environ. Technol.*, **1993**, *14*, 851-859.

Fanchaing, Y. T.; Wood, J. M., *J. Am. Chem. Soc.*, **1981**, *103*, 5100-5103.

Forsyth, D.S.; Hayward, S., *Fresenius J. Anal. Chem.*, **1995**, *351*, 403-409.

Forsyth, D. S.; Cleroux, C., *Talanta*, **1991**, *38*, 951-957.

Francois, R.; Short, F. T.; Weber, J. H., *Environ. Sci. Technol.*, **1989**, *23*, 191-196.

Francois, R.; Weber, J. H., *Mar. Chem.*, **1988**, *25*, 279-289.

Gilmour, C. C.; Tuttle, J. H.; Means, J. C., *Microb. Ecol.*, **1987**, *14*, 233-242.

Gilmour, C. C.; Tuttle, J. H.; Means, J. H., *Anal. Chem.*, **1986**, *58*, 1848-1852.

Gilmour, C. C.; Tuttle, J. H.; Means, J. C., In "Marine and Estuarine Geochemistry"; Sigleo, A. C.; Hattori, A. (Eds.); Lewis Publ., Inc.: Chelsea, MI, USA, **1985**.

Hall, L. W.; Pickney, A. E., *CRC Critica.l Reviews in Toxicology*, **1985**, *14*, 159-209.

Hallas, L. E.; Means, J. C.; Cooney, J. J., *Science*, **1982**, *215*, 1505-1507.

Hamasaki, T.; Masumoti, H.; Sato, T.; Nagase, H.; Kito, H.; Yoshioka, Y., *Appl. Organomet. Chem.*, **1995a**, *9*, 95-104.

Hamasaki, T.; Nagase, H.; Yoshioka, Y.; Sato, T., *Crit. Reviews Environ. Sci. Technol.*, **1995b**, *25*, 45-91.

Hamasaki, T.; Nagase, H.; Sato, T.; Kito, H.; Ose, Y., *Appl. Organomet. Chem.*, **1991**, *5*, 83-90.

Han, J. S.; Weber, J. H., *Anal. Chem.*, **1988**, *60*, 316-319.

Hines, M. E.; Knollmeyer, S. L.; Tugel, J. B., *Limnol. Oceanogr.*, **1989**, *34*, 578-590.

Huang, G.; Bai, Z.; Dai, S.; Xie, Q., *Appl. Organomet. Chem.*, **1993**, *7*, 373-380.

Jackson, J. A.; Blair, W. R.; Brinckman, F. E.; Iverson, W. P., *Environ. Sci. Technol.*, **1982**, *16*, 110-119.

Kennedy, J. D., *J. Labelled Compounds*, **1975**, *11*, 285-286.

Kon, H.; Serizawa, S.; Katsu, M.; Morita, M., *Anal. Sci.*, **1991**, *7 (supplement)*, 1065-1068.

Krull, I. S.; Panaro, K. W., *Appl. Spectros.*, **1985**, *39*, 960-968.

Lawson, G.; Ostah, N., *Appl. Organomet. Chem.*, **1993**, *7*, 183-191.

Le, X.-C.; Cullen, W. R.; Reimer, K. J.; Brindle, I. D., *Anal. Chim. Acta*, **1992**, *258*, 307-315.

Lee, D. S.; Weber, J. H., *Appl. Organomet. Chem.*, **1988**, *2*, 435-440.

Lytle Jr., R. W.; Hull, R. J., *Agron. J.*, **1980**, *72*, 942-946.

Maguire, R. J., *Water Pollut. Res. J. Ca.n.*, **1991**, *26*, 243-360.

Maguire, R. J.; Tkacz, R. J.; Chau, Y. K.; Bengert, G. A.; Wong, P. T. S., *Chemosphere*, **1986**, *15*, 253-274.

Manders, W. F.; Olsen, G. J.; Brinckman, F. E.; Bellama, J. M., *J. Chem. Soc. Chem. Commun.*, **1984**, 538-540.

Martin, F. M.; Donard, O. F. X., *Fresenius J. Anal. Cham.*, **1995**, *351*, 230-236.

Means, J. C.; Hulebak, K. L. *Neuro Toxicology* **1983**, *4*, 37-44.

Miller, J. C.; Miller, J. N., "Statistics for Analytical Chemistry", 2nd ed.; Halsted Press: New York, **1988**.

Minganti, V.; Capelli, R.; De Pellegrini, R., *Fresenius J. Anal. Chem.*, **1995**, *351*, 471-477.

Quevauviller, P.; Lavigne, R.; Pinel, R.; Astruc, M., *Environ. Pollut.*, **1989**, *57*, 149-166.

Randall, L.; Donard, O. F. X.; Weber, J. H., *Anal. Chim. Acta*, **1986a**, *184*, 197-203.

Randall, L.; Han, J. S.; Weber, J. H., *Environ. Technol. Lett.*, **1986b**, *7*, 571-576.

Rapsomanikis, S.; Donard, O. F. X.; Weber, J. H., *Anal. Chem.*, **1986**, *58*, 35-38.

Rapsomanikis, S.; Weber, J. H., *Environ. Sci. Technol.*, **1985**, *19*, 352-356.

Ring, R. M.; Weber, J. H., *Sci. Tot. Environ.*, **1988**, *68*, 225-239.

Seligman, P. F.; Valkirs, A. O.; Stang, P. M.; Lee, R. F., *Mar. Pollut. Bull.*, **1988**, *19*, 531-534.

Shea, M. L.; Warren, R. S.; Niering, W. A., *Ecology*, **1975**, *56*, 461-466.

Short, F. T., Ed. "The Ecology of the Great Bay Estuary, New Hampshire and Maine: An Estuarine Profile and Bibliography"; NOAA Coastal Ocean Program Publ.:, **1992**.

STATA Reference Manual, Computing Resource Center, **1990,** 529-530.

Szpunar-Lobinska, J.; Witte, C.; Lobinska, R.; Adams, F. C., *Fresenius J. Anal. Chem.*, **1995**, *351*, 351-377.

Thompson, J. A. J.; Sheffer, M. G.; Pierce, R. C.; Chau, Y. K.; Cooney, J. J.; Cullen, W. R.; Maguire, R. J., "Organotin Compounds in the Aquatic Environment: Scientific Criteria for Assessing their Effects on Environmental Quality"; National Research Council of Canada: Ottawa, **1985**.

Tugrul, S.; Balkas, T. I.; Goldberg, E. D., *Mar. Pollut. Bull.*, **1983**, *14*, 297-303.

Weber, J. H.; Billings, M. R.; Falke, A. F., *Est. Coast. Shelf Sci.*, **1991**, *33*, 549-357.

Weber, J. H.; Alberts, J. J., *Environ. Technol.*, **1990**, *11*, 3-8.

Weber, J. H.; Han, J. S.; Francois, R., In "Heavy Metals in the Hydrological Cycle"; Astruc, M.; Lester, J. N., Eds.; Selper, Ltd.: London, **1988**.

Wright, P. J.; Weber, J. H., *Environ. Sci. Technol.*, **1991**, *25*, 287-294.

Wilkins, R. G., in *The Study of Kinetics and Mechanism of Reactions and Transition Metal Complexes,* Allyn and Bacon, Boston, **1974**, 16-19.

APPENDIX

STATISTICS

The copious amounts of data gathered and analyzed in these experiments make analysis very tedious and complicated, if not entirely impossible without the use of statistics. Statistical tests were employed to indicate the significance of any particular variable in an experiment. The computer program used for statistical analysis in this research was STATA. Explanations of the statistical tests used in this reasearch follows. All statistical methods determine the probability that the null hypothesis is true. In other words, the tests assume that the data sets are NOT different and ascertain to what degree that is true.

Parametric Tests

Parametric tests are used when the data conform to a normal Gaussian distribution and the variance is homogeneous. They are more powerful than non-parametric tests, and therefore, favored if the data meets the assumptions of normality. The central limit thorem states that even if the original population is not normal, the sampling distribution of the mean tends to a normal distribution as the population size increases (Miller and Miller, 1988). In all cases contained in this research, the sample size is small (less than 5) and the distribution of the chosen population was not normal, but there is every reason to believe that with

increasing population size, the distribution would be Gaussian. In these cases, based on the central limit theorem, parametric statistics may be used.

An analysis of variance (ANOVA) is used to compare several means. It separates the variation in measurement due to the controlled factor from that due to random error. For example, in the seasonal experiment (Weber *et al.*, 1991) (Chapter 2), samples were analyzed for methyltin concentration every week for several weeks. In this example, there were 2 possible sources of variation in the measurements. The first arises from random error, which is impossible to avoid in analytical measurements. The second, which is of much greater interest to the experimenter, is due to any real difference occuring over time. ANOVA is used in this case to determine if, after the variation due to random error has been accounted for, there is a significant temporal difference in the mean concentrations.

ANOVA begins with the assumption that all samples are drawn from the same population (Miller and Miller, 1988). If this is true, the variation (σ^2) between different measurements of the same sample will be the same as the variation among different samples. The within sample estimate of variance is determined by:

$$\sigma^2 = \Sigma_i\Sigma_j (x_{ij} - \bar{x}_i)^2 / h(n-1) \qquad \text{(Equation A.1)}$$

where x_{ij} is the *j*th measurement of the *i*th sample, $\bar{x}_i$ is the sample mean, n is the number of runs of each sample, and h is the number of different samples. Summation over *j* and

division by n-1 gives the variance of each sample and summation over *i* and division by h averages sample variances. The between sample estimate of variance is calculated from:

$$\sigma^2 = n\Sigma_i(\bar{x}_i - \bar{x})^2 / (h-1) \qquad \text{(Equation A.2)}$$

where $\bar{x}$ is the overall mean. ANOVA compares the 2 estimates of variance to determine if they differ significantly.

A two-way ANOVA can be used when more than one controlling factor is altered. This gives the added advantage of being able to separate variations due to each of the controlling factors, to any interaction between controlling factors, and to random error (STATA Reference Manual, 1990).

Rejection of the null hypothesis by ANOVA can occur under several circumstances. One sample may differ significantly from all others within the group, each sample may differ significantly from every other sample, or a range of possibilities between those extremes. Further testing must be used to ascertain the reason for a significant result. One method is to look at the underlying regression coefficients. By using the 'regress' command in STATA® each sample mean is compared individually to a preselected sample mean (STATA). The regression table indicates wether the individual mean is significantly less than, significantly greater than, or not significantly different from the preselected sample mean. By performing

this function several times, choosing a different sample to compare all others to each time, a complete picture can be obtained.

Non-parametric Tests

Often in environmental work, there is either insufficient data to invoke the central limit theorem or, especially when working in trace analysis, the data is positively skewed with the majority of the measurements falling around the limit of detection and some below detection. Representing a data set of this sort with a mean and standard deviation is inaccurate. Non-parametric analysis allows statistical testing without assuming anything about the distribution. While these tests are more robust than parametric tests, they suffer in that they are not as powerful.

In a seasonal study conducted by Falke and Weber (1993) (Chapter 3), there was a need to compare the MeSn concentrations in 4 different parts of the plant to each other. This comparison was confounded by a time variable as the samples were collected on a weekly basis and weekly differences had to be taken into account. Also, since only 2 subsamples of each sample type were analyzed, a normal distribution could not be assumed. The Kruskal-Wallis test can be used to compare 3 or more data sets of paired results (Miller & Miller, 1988). Basically, this test is used to determine whether there is a consistent enough order to the ranking of the data sets to suggest that they do not all come from the same population. For each set of paired data, ranks are assigned so that the lowest value is given the rank

number 1 and the next larger value is assigned 2 and so on. Tie values receive average ranks. The ranks for each data set are then summed and the Chi squared (χ^2) statistic is used to assess the differences that occur between the total rank values:

$$\chi^2 = 12R/[nk(k + 1)] - 3n(k + 1) \qquad \text{(Equation A.3)}$$

where R is sum of the square of the rank sums, n is the number of samples, and k is the number of data sets. Once the acceptable confidence level has been determined, the critical value for χ^2 is determined from statistical tables. Comparison of the determined value for χ^2 with the tabulated critical value indicates whether the data sets are significantly different.

If a significant difference is indicated when all samples are considered together, separate tests may be used to identify where the differences occur. In the example used above, the Friedman's test indicated that the samples were not all the same. At this point, the Wilcoxon Signed Ranks Test will help to determine whether 2 different plant parts have significantly different concentrations of MeSn.

The Wilcoxon Signed Rank Test may be used as a non-parametric alternative to the paired T-test. It tests the equality of distributions for matched pairs of observations. If there is no difference between the 2 sets of data, the differences between them will be symmetrically distributed around zero. To determine if this is so, the differences between the 2 paired data points are arranged in ascending order without regard to sign and assigned a numerical rank,

retaining the original sign. Tied values are both assigned the average rank. Positive and negative rank values are added and the lowest absolute value is the test statistic. Tabulated values indicate the probability of this particular value occurring. Comparison of this value to the tabulated value at the desired level of confidence indicates the significance of the difference between the 2 data sets. If the calculated test statistic is lower than the tabulated value, the null hypothesis is rejected.

In conclusion, it must be stressed that statistics, while extremely useful to the researcher, are only tools. They cannot be use to substitute for knowledge, only to support it. They do not divide environmental data into a definitive world, but, rather leave many nebulous areas to be explained by the researcher. It is with this awareness that statistics have been applied throughout these experiments.